

# Microbiology

## Handwritten Note

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Name: \_\_\_\_\_

Subject: \_\_\_\_\_

Microbiology



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# BACTERIAL VIRULENCE FACTORS

17/2/18

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Responsible for pathogenesis

Vaccine producer is also by virulence factors

Q. Smooth → virulent strain → so these are used for vaccines  
Rough → avirulent strain

Q. All of the following can be used as vaccine to prevent E. coli diarrhoea except

a) CS2

b) K88

c) CFA

d) P<sub>1</sub>

All are fimbriae

a, b, c show adhesion to intestinal epithelial cells

d shows adhesion to uroepithelial cells.

## FIMBRIAE (PILI)

- They are glycoprotein

- Antigenic

- agglutinate RBC

- Nomenclature is based on the RBC agglutinated by RBC.

Function:-

1> Adhesion (Gram -ve)

In Gram +ve → adhesion is due to Teichoic Acid

2> Conjugation.

Mechanism of transfer of genetic material

They are Plasmid encoded.

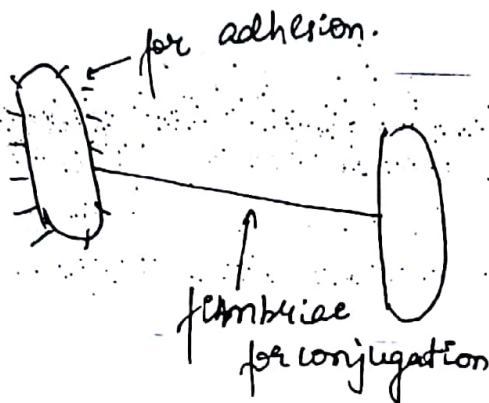
F<sup>-</sup> bacteria can't initiate conjugation

Enterococcus conjugates out fimbriae.

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Plasmid encoded fimbriae

- Mannose resistant fimbriae
- CFA (colonising factor antigen) [ETEC]
- Type 1 fimbriae



## FLAGELLA

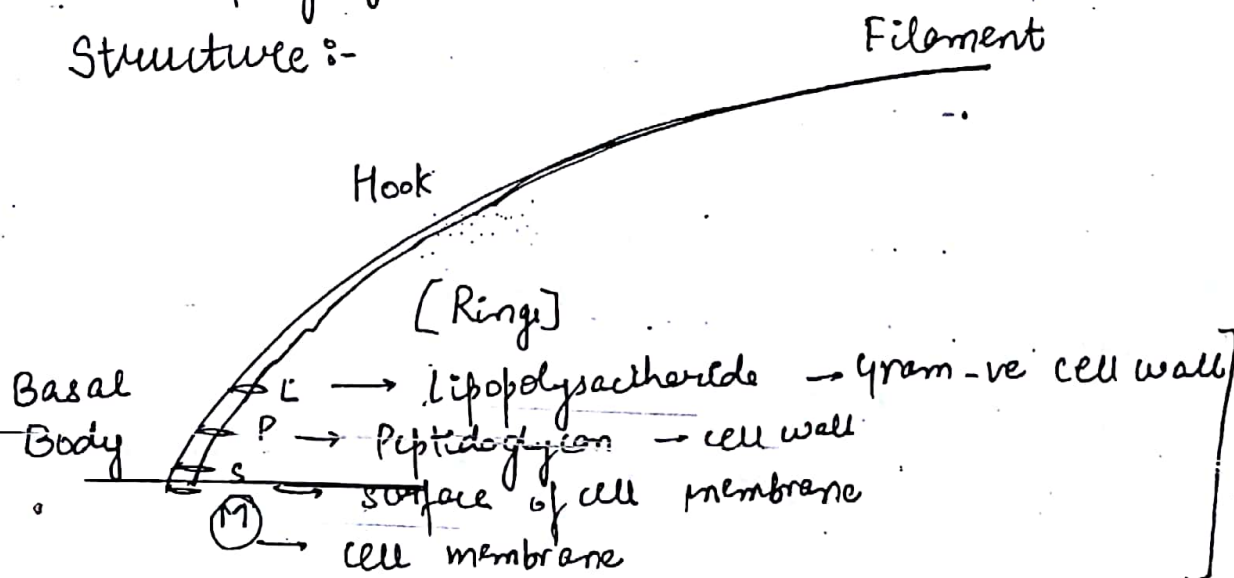
- Protein

- antigenic 'H' Ag
- Thin  $< 0.02 \mu\text{m}$

Function:-

- 1) Motility - due to rotation of M Ring
- 2) Antiphagocytosis

Structure:-



In Gram -ve → all rings are found 5

In Gram +ve → only S.M. rings

↓  
only cell membrane attachment

Motility helps in identification of bacteria

~~Flao~~

Resolution Power

naked eye → 200  $\mu\text{m}$

Light microscope →  $\frac{200}{1000} \mu\text{m} = 0.2 \mu\text{m}$   
 $= 200 \text{ nm}$

Electron microscope =  $\frac{200 \text{ nm}}{1000} = 0.2 \text{ nm}$

Flagella can't be seen by light microscope.

Demonstration of Flagella/Motility:-

→ Electron microscope

→ Ryu stain  
silver based stain.  
not sensitive

→ Serology.  
H antigen.

→ Hanging Drop (for vibrios only)

→ Dark ground microscope (spirochaetes)

Best Method Growth in semisolid agar (Motility medium)  
↓  
0.2 to 0.5% agar

Peritrichous Flagella-

flagella all round

Enterobacteriaceae

Lophotrichous Flagella-

tuft of flagella at one end.

~~can~~ campylobacter

Helicobacter

Lophase camp m<sup>e</sup> Halla

Single polar

Vibrio

Pseudomonas.

Swarming can be inhibited by all except —

a) 6% agar

b) phenol agar

c) blood agar

d) McConkey agar

### Culture Media

Liquid

Solid

Basal media → Nutrient broth

agar 1-2%

Peptone

Meat extract

NaCl } 0.85N

H<sub>2</sub>O

Buffers

solidifying temp 42°C

⊖ flagellin

except

Proteus }  
A. Tetani }  
B. cereus }

swarming



Inhibition of swimming → 6% agar  
Phenol

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Boric Acid

Bile salts (tnt in McConkey)

Craig's tube contain semisolid media but ~~not~~ not  
<sup>much</sup> used for motility.

But in case of solid, semisolid media, it can be  
preferred for motility.

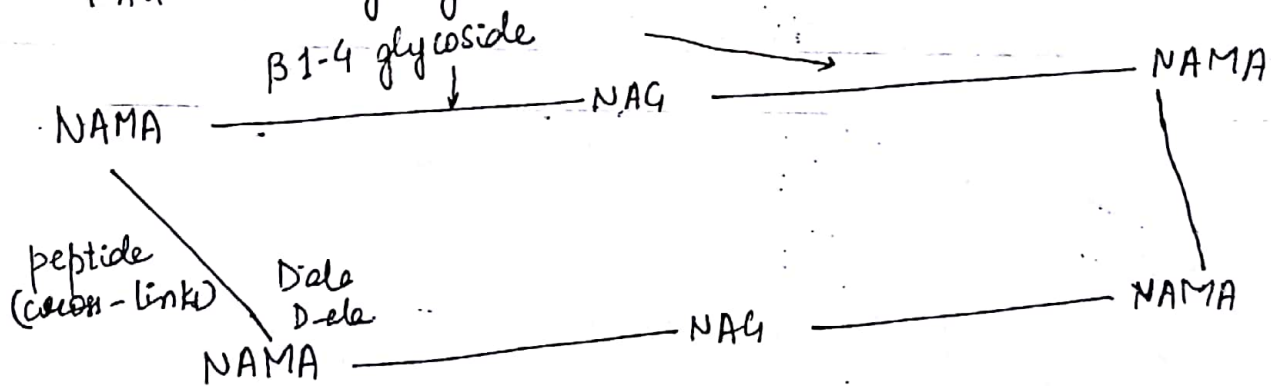
## CELL WALL

Peptidoglycan

Structure

NAMA → N-acetyl muramic acid

NAG → N-acetyl glucosamine



$\text{COO}^-$  terminal in NAMA has unsaturated ends, so  
form bonds while NAG doesn't have this.

Synthesis:-

Peptidoglycan is synthesised by **PBP** (penicillin Binding protein)  
↓  
located in cell membrane

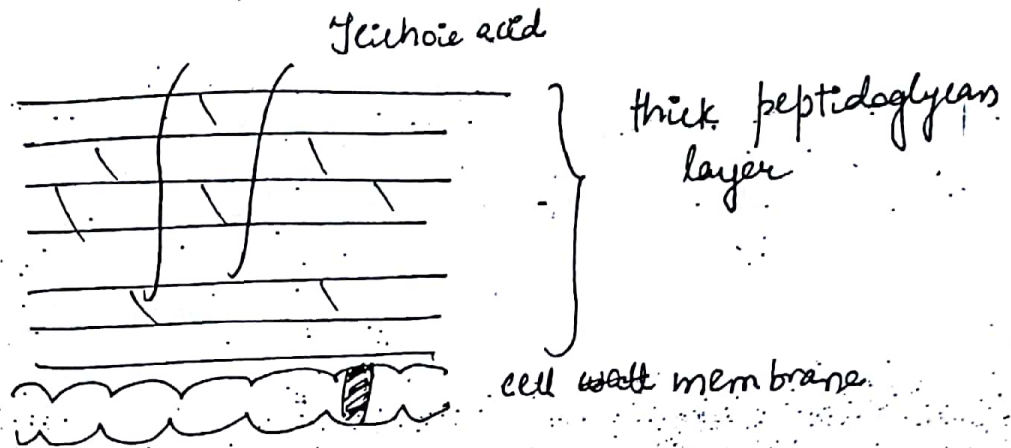
Cross-linking (4<sup>th</sup> Phase → last phase)



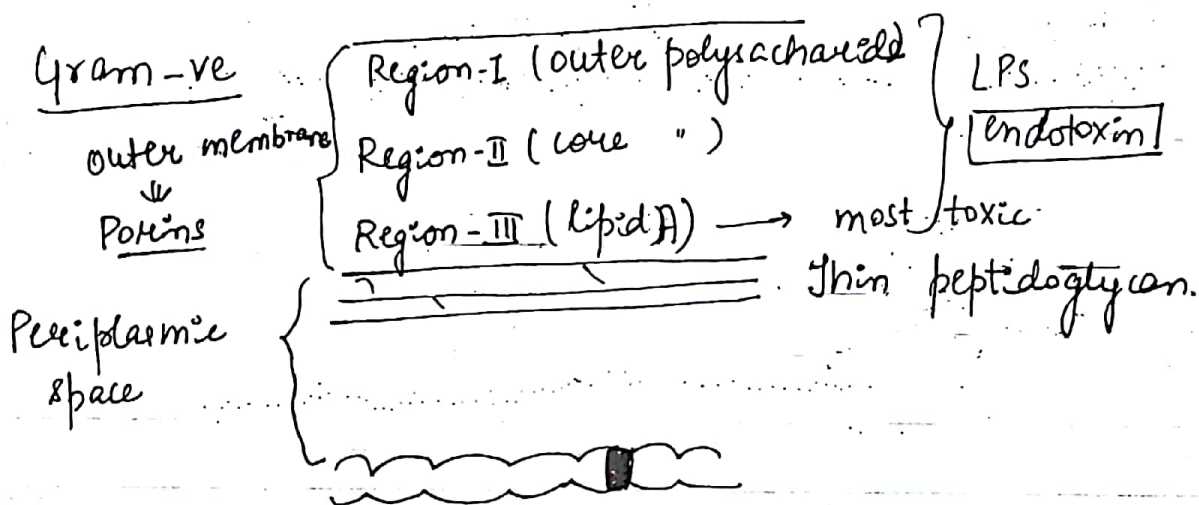
# Types of cell wall

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Gram +ve

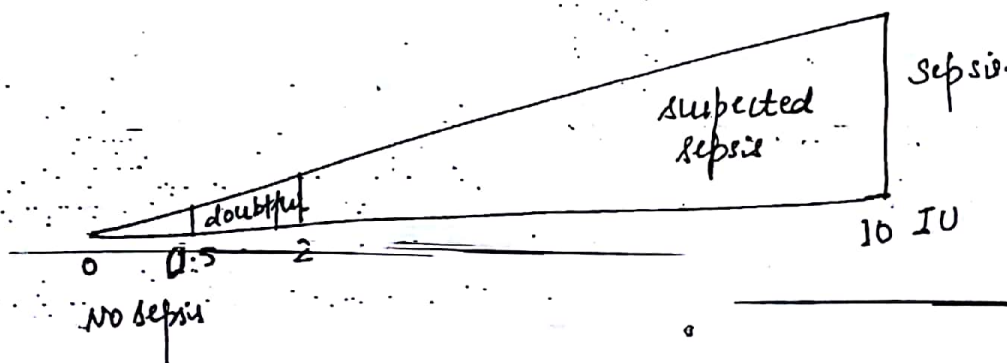


Gram -ve



Lipid A → binds to Toll like Receptor 4 on macrophages.  
(most toxic)

Best marker of Sepsis = Pro calcitonin



LPS → Hydrophobic

Pore helps in passive diffusion of hydrophilic agents

Region I (outer polysaccharide) is antigenic

It is called as O' antigen

O' Antigen

→ useful for diagnosis (immunogen)

→ Serological ⇒ Serotyping (variability of O' antigen)

↓  
epidemiology Q.

Cholera endotoxin doesn't cause virulence

Acid Fast Cell Wall :-

Gram +ve cell wall = Mycolic acid

Function:-

Rigidity (cell wall)

CAPSULE

- Polysaccharide.

except B. anthracis → made up of polypeptide  
(D-Glutamic acid) Q.

- Polysaccharides are not stained.

But capsule of B. anthracis can be stained by

Polychromatic Methylene Blue (McFadyean Reaction)

Function:

Antiphagocytosis Q.

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Demonstration of capsule

1) M/I  $\Rightarrow$  Negative stain  $\rightarrow$  India Ink  
or  
Nigrosin

2) Serology  $\rightarrow$  Quellung Rx  
swelling of capsule using capsular Ab.  
 $\downarrow$   
Microscope

b) capsular Ag detection by Latex agglutination

$\downarrow$   
Reverse passive agglutination

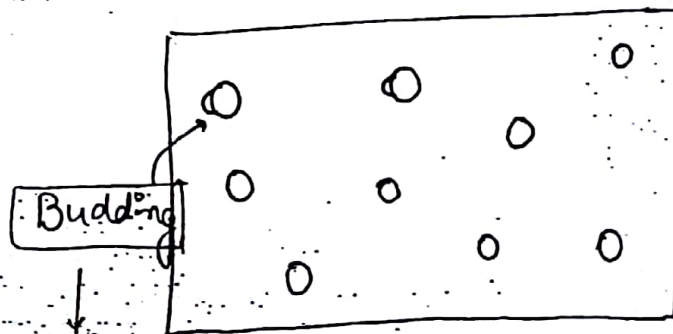
$\downarrow$   
when Ag is detected, then it is called reverse passive.

$\downarrow$   
when particles are used for Ab detection.  
eg. Latex  
RBCs

Q. A pt. presented  $\bar{c}$  headache, projectile vomiting along  $\bar{c}$  altered sensorium. Image of india ink.

$\Delta$

- a) Cryptococcus
- b) Pneumococcus
- c) Histoplasma
- d) Coccidioides



seen in Cryptococcus

pneumococcus show Lancelet shaped.

Diplococci

Gram -ve → Neisseria

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Gram +ve → Pneumococci

Neisseria (Intracellular Diplococci)

Meningococci  
capsulated

Gonococci

uncapsulated  
kidney shaped

serological assay on microscope slides are not  
done except VDRL

Quellung Reac.

↓  
Non-specific test

Polyvalent antepolysaccharide serum

Capsulated Organisms

Yes the PM cooks Very ~~like~~ Fine Chicken to keep his  
BBB healthy

- Yersinia
- Pneumococcus
- Meningococcus
- Cryptococcus
- Vibrio parahaemolyticus
- Vibrio cholerae O<sub>139</sub> Q

- High strains of E. coli, staph, strep, pseudomonas

↓  
Nosocomial (HAI)

- Clostridium perfringens & Butyrium



Klebsiella

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- B. anthracis

- Bordetella

- Bacteroid

- Burkholderia pseudomallei

- Haemophilus influenzae

\* Histoplasma capsulatum

✓ Non-capsulated.

✓ intracellular yeast & appear as capsulated

## ENZYMES & TOXINS

Secreted by Secretion System.

→ Seven Pathways - type I to VII

Type I & IV - Both in Gram +ve & Gram -ve

Type II, III, V, VI → only in Gram -ve

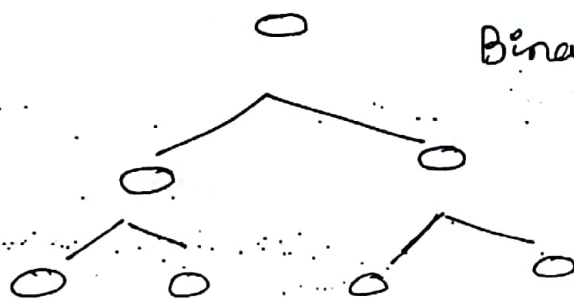
Type VII → M. TB.

→ Proteins secreted by Type I, III, & VI pathways - transverse inner membrane & outer membrane in one step  
(see independent - Do not involve amino terminal processing of secreted protein)



# Growth Curve

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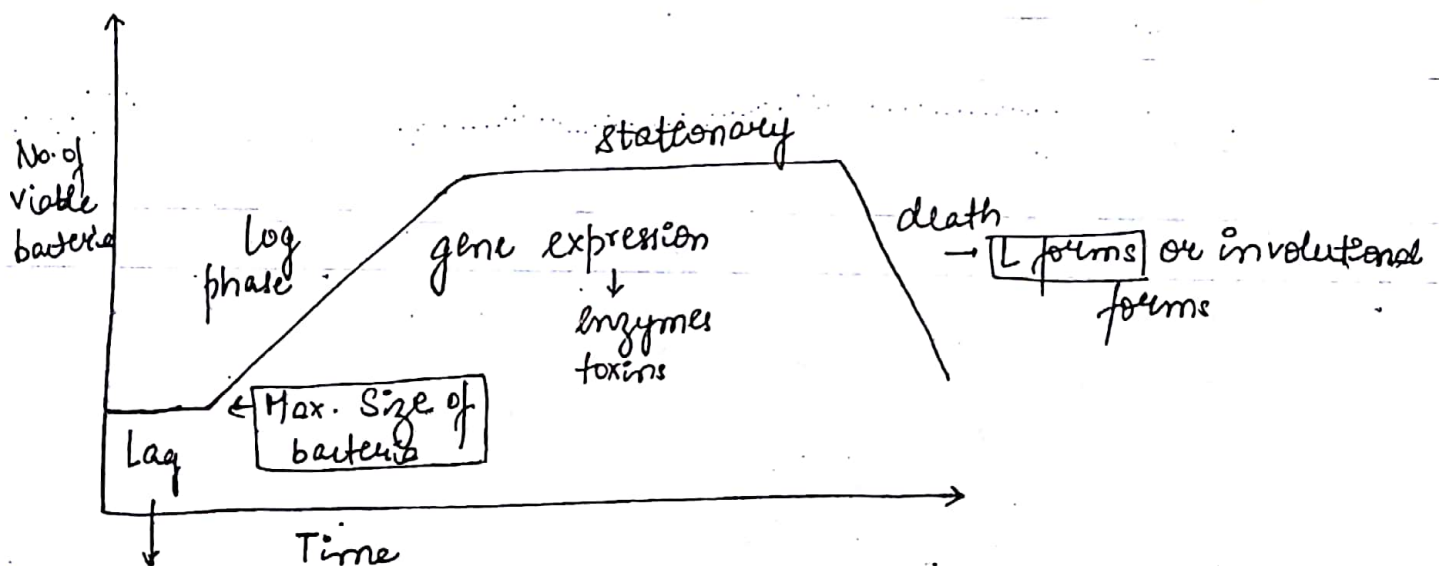
Binary fission (asexual)

Doubling Time is called Generation Time

Max generation Time seen in *M. Leprae* - 13-14 days

*M. Tb* → 16 hrs

other bacteria → 20 min. H/c



preparatory phase

↓  
enzymes are released  
[Metabolic Phase]

Ante microbial susceptibility is done by Phenotypic Method  
↓  
in lag phase

→ Stationary phase is due to toxic metabolic products  
→ bacterial colony form → exhaustion of nutrients  
on solid medium

\* Sporulation is seen in stationary phase. & this is for survival.

They are highly resistant bcoz they have  
Ca dipicolinic acid synthetase

↓  
↓ absorption of water  
↓  
↑ Resistance

Δ - 0.5% H<sub>2</sub>SO<sub>4</sub> → Zn stain

L forms -

- No cell wall

- Mycoplasma

- Spheroplast → Gram -ve organisms  
incomplete destruction of cell wall.

↓  
Reversible

- Protoplast → Gram +ve organism  
complete destruction of cell wall

↓  
Irreversible

- L-forms are virulent

P → +ve

Q. A 25 year old lady presents  $\bar{c}$  ↑ frequency & dysuria  $\bar{c}$  from  $\ominus$  bacilli in urine.

After 2 weeks of penicillin t/t she comes back w/out resolution of her complaints?

$\bar{c}$  Gram -ve cocci.

a)  $\beta$  lactamase producing strain

b) Gonococci

c) Sphaeroplast

d) protoplast

R<sub>x</sub> - Discontinue R<sub>x</sub> for 24-48 hr. + ~~test the drug~~  
Start the drug  $\bar{c}$  proper dosage

# BACTERIAL GENETICS

## Prokaryotes

↳ No membrane bound organelle.

(no nu, mitochondria, no ER, golgi complex)

↓

Respiratory func<sup>n</sup> → mesosomes.

(invagination of cell membrane)

No histone proteins.

↓

packaging of DNA → supercoiling

Genes are located in chromosome

Plasmid,

Transposon.

Chromosome, Plasmid → ds DNA

circular

self replicating

↓

DNA polymerase.

Plasmid are extra-chromosomal.

↓

'No metabolic func<sup>n</sup>'

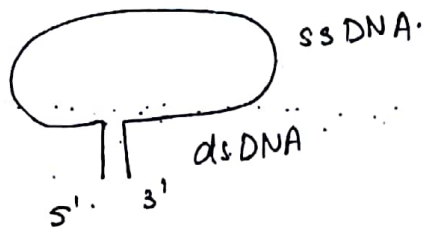
Mobile

# Mobile Genetic Transposon Elements

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## ① Transposon

oligonucleotide stranded  $\bar{c}$  complementary ends.



carry gene  $\rightarrow$  impart new character

Transposons move in cut & paste manner bind to

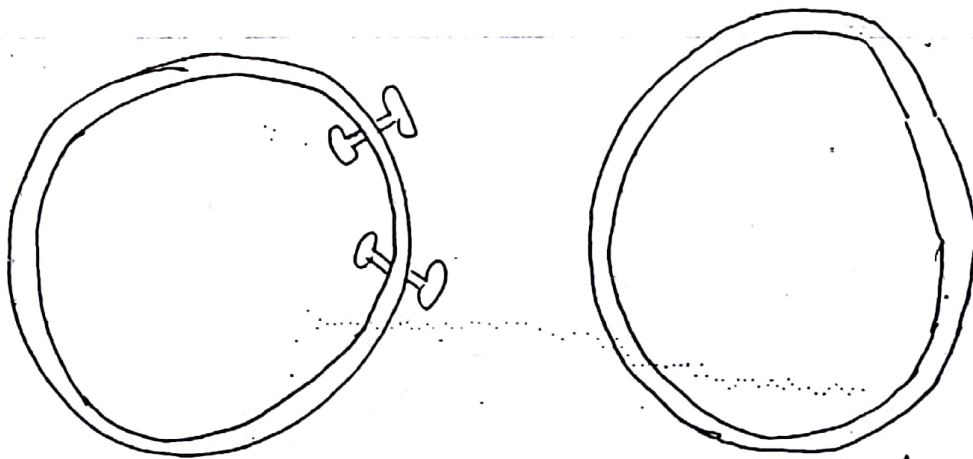
tRNA

Jumping Gene

Insertion is not complementation.

insert directly between nucleotides.

Repeat  
Sequence  
formation



Plasmid mediated drug resistance is due to insertion of Transposons.



## ② Insertion Sequence (IS)

Similar to transposon (smaller)

18

No gene (phenotypic silence)

↓  
using this Genotyping can be done

Gold std for MTB for genotyping is IS 6110 typing  
This is not for M. Bovis

M. Bovis - Spoligotyping  
(spacer oligonucleotide) → we see here repeats

## TRANSFER OF GENE

seen in bacteria

✓ Transformation

✓ Transduction

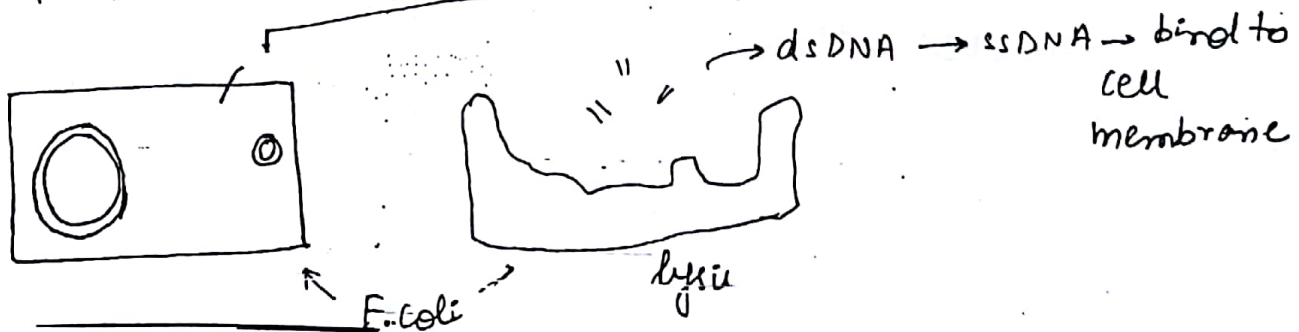
✓ Conjugation

## TRANSFORMATION

Transfer of gene from 1 bacteria to another by  
naked DNA

↓

fragment of DNA after lysis of bacteria



## Homologous Recombination

Replacement of a part of 1 strand in bacterial<sup>19</sup> genome by <sup>ss</sup>naked DNA fragment at similar genes or alleles

Greeffith - Live type II non-capsulated Pneumococci (R)

↓  
injected into mice → no pathogenicity

(+)

Killed Type I capsulated Pneumococci (S)

↓  
injected into mice

↓  
no pathogenicity

When both were mixed together & injected into mice

↓  
Death

↓  
Bacteria is cultured

↓  
Live Type I capsulated pneumococci

He coined the term Transformation.

This was called ~~For~~ Pioneering experiment in genetics.

Transformation is used in Recombinant DNA Technology

→ Antigens

→ Vaccines

Drug resistance

Transformation may lead to ~~to~~ drug resistance.

## TRANSDUCTION

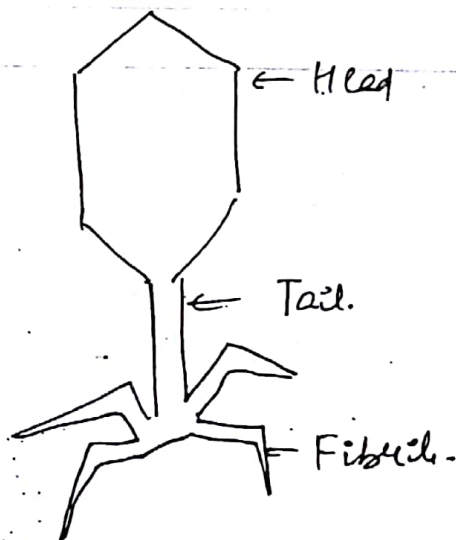
MLC method of gene transfer

Transfer of genes from 1 bacteria to another by

Bacteriophage



virus infecting bacteria



RNA phage



filamentous

DNA phage can be used for bacterio-  
phage typing

Q ☐ phage do not carry out transduction?

a) Lytic

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b) Lysogenic

c) temperate

d) lambda

Q.  $\phi$  phage shows lysogenic to lytic phase conversion?

as ① Temperate phage i lambda

Q.  $\phi$  phage do not show lysogenic to lytic phase conversion?

Lysogenic phage

Q. Genes associated  $\bar{c}$  Galactose metabolism is transferred by  $\phi$  phage

Lambda phage

Q. Sex conversion in salmonella is

a) transformation

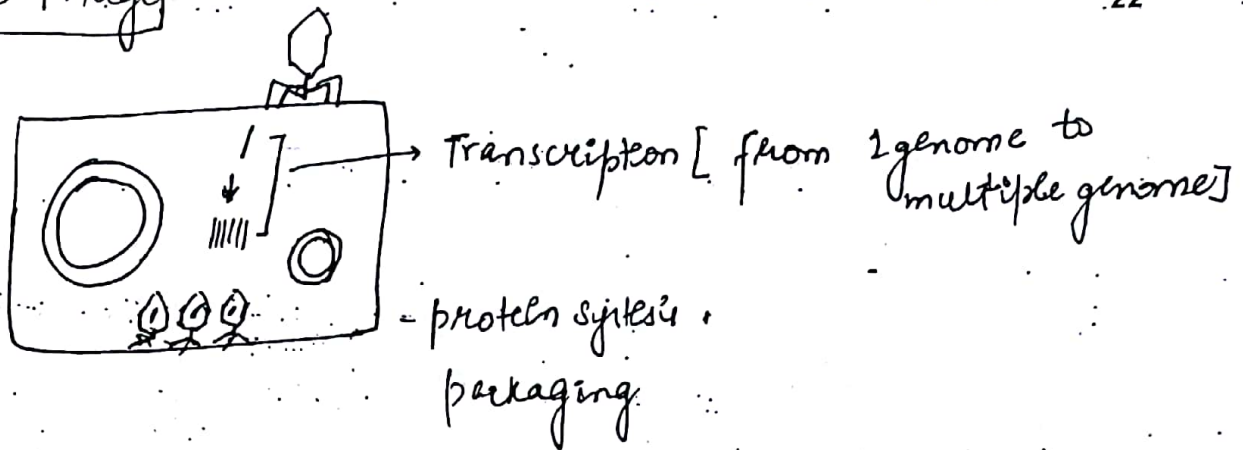
b) transduction

c) Lysogenic

d) conjugation

# Lytic Phage:

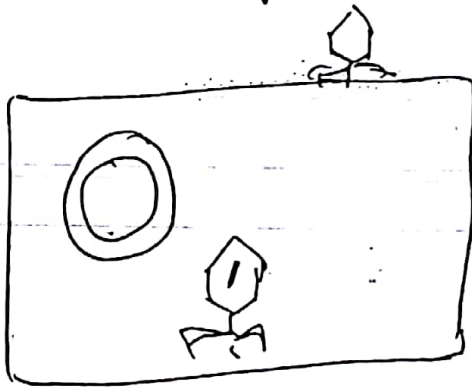
22



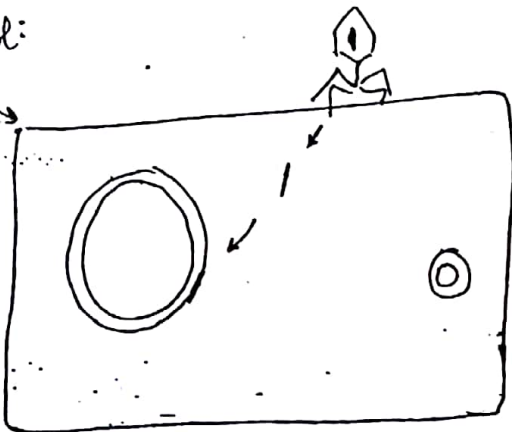
No transduction occurs normally → if error is not there

In case of error while packaging, if bacterial genome enters ~~viral~~ viral —

↓  
It goes to other bacteria → inserts the —  
transfer the gene to  
this bacteria.



E. Coli:





## Generalized Transduction :-

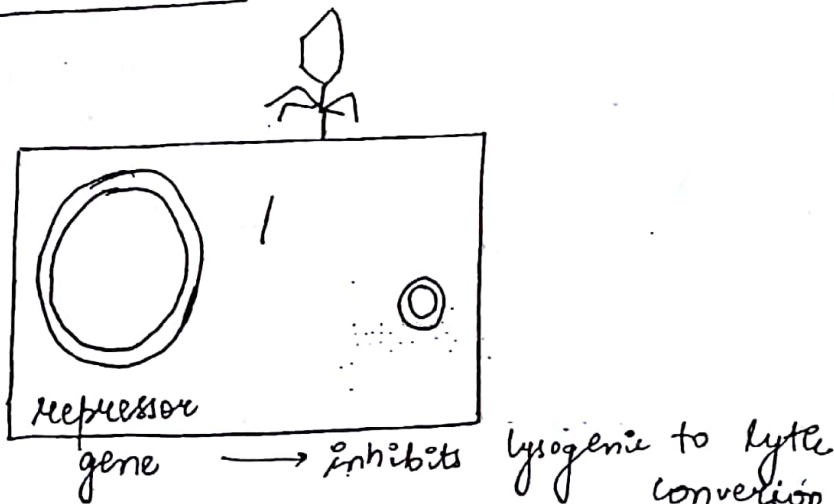
23

- lytic phase
- error in packaging
- Homologous recombination
- every ~~the~~ gene has equal chance of transfer

## Lysogenic Phage :- NO Transduction

- Incorporation of phage DNA ~~to~~ into bacterial genome  
by homologous recombination  
↓  
Lysogeny [Transfer from viral to bacteria]

- Rare phenomenon
- Seen in certain bacteria
- Imparts new character to the bacteria
- No lysogenic to lytic phase conversion.  
↓
- NO transduction.



Repressor gene is stimulated by lysogenic

eg:-

24.

## 1> Seroconversion in Salmonella

S. anatum O<sub>3, 10, 12</sub> H<sub>6, i.e.</sub>

↓  
Phage 15

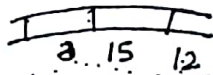
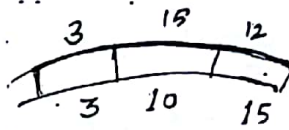
S. Newington

↓  
Phage 34

S. Minnesota

O<sub>3, 15, 34, 12</sub> H<sub>6, i.e.</sub>

ANM



for serotyping → use monovalent 'O' antiserum.

for Polyvalent is used for identification of bacteria as a whole (eg. salmonella)

## 2> Corynebacterium Diphtheriae

β phage → tox gene

↓  
'toxin'

due to lysogenic phage, β phage imparts tox gene into corynebacterium leading to toxin production. then it gets released toxin, hence "pathogenic".

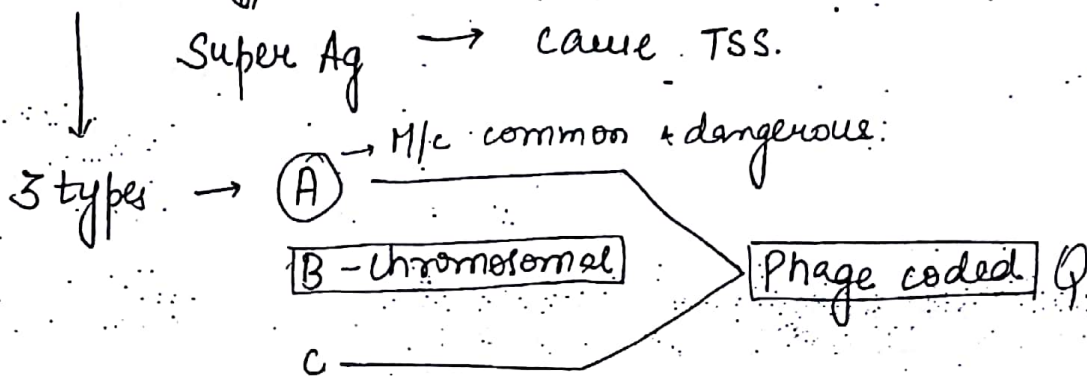
↓

Hence we do toxigenicity test in Lab Δ of corynebacterium.

### 3> Gr. A Streptococcus (S. pyogenes)

25

Pyrogenic exotoxin (Erythrogenic toxin)



Scarlet fever.

TSS in Group A streptococcus leads to scarlet fever.

If lysogeny occurs → ~~severe~~ severe scarlet fever

Scarlet fever without lysogeny → mild

4> V. cholerae

5> Shigella

6> C. botulinum

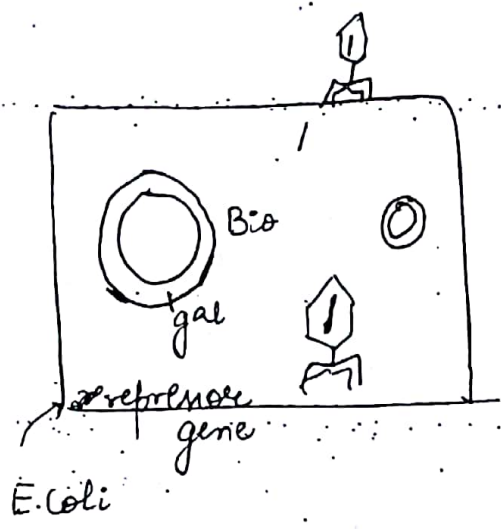
exotoxins.

Temperate Phage ⇒ Transduction (+)

They show Lysogenic to Lytic conversion

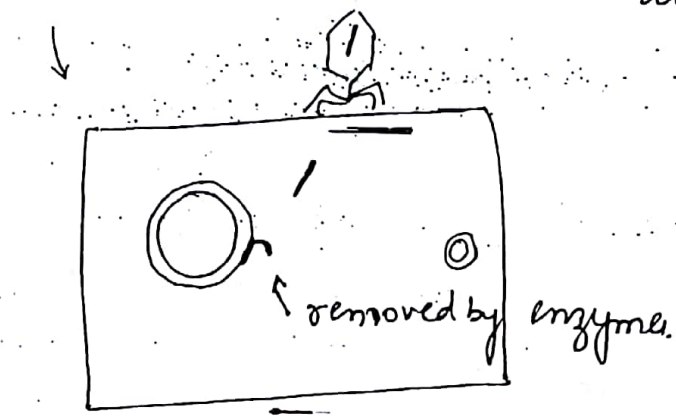
eg. Lambda phage → insert phage DNA bet<sup>n</sup> gal + bio genes

↓  
Galactose metabolism



when bacterie starts dying.  
(repressor gene stops acting)

↓  
Phage. pusten are formed,  
genetic material is taken out  
↓  
due to error bacterial gene  
are also detached along  
viral genome (error in  
excision)



↓  
~~viral~~ Phage infects other  
E. coli  
↓

Specialized or restrictive  
transduction

But due to more  
similarity to bacterial genome  
only bacterial genome is  
inserted into this  
bacteria & viral  
genome is removed  
by enzymes

- Temperate phage
- error in excision
- Homologous recombination  
seen

- specific genes transferred

↓  
Lambda → genes associated  
galactose metabolism

Transduction



Transduction also can lead to Drug Resistance

## CONJUGATION

Transfer of genes from 1 bacteria to another by Plasmid

Q. Transfer of chromosomal genes by conjugation is seen in-

a)  $F^+$

b)  $F^-$

c)  $Hfr$

d)  $F'$

PGI -  $Hfr$ ,  $F'$

NBE -  $Hfr$ .

If 1  $Hfr$  (cell 1) having A, B, C & D genes after the plasmid conjugate  $\bar{c}$  100  $F^-$  bacteria. (cell 2) then predominant genotype would be

a) cell 1  $\bar{c}$  A, B, C, D

b) cell 1  $\bar{c}$  A

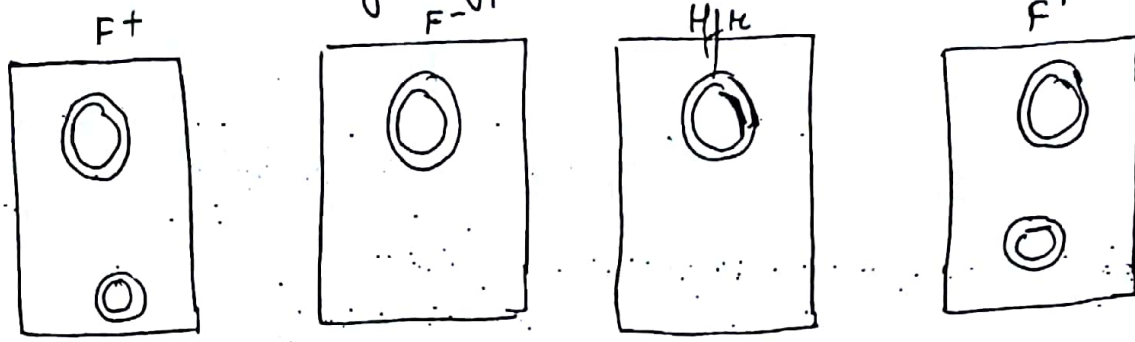
c) cell 2  $\bar{c}$  A, B, C, D

d) cell 2  $\bar{c}$  A

Since no change in genotype

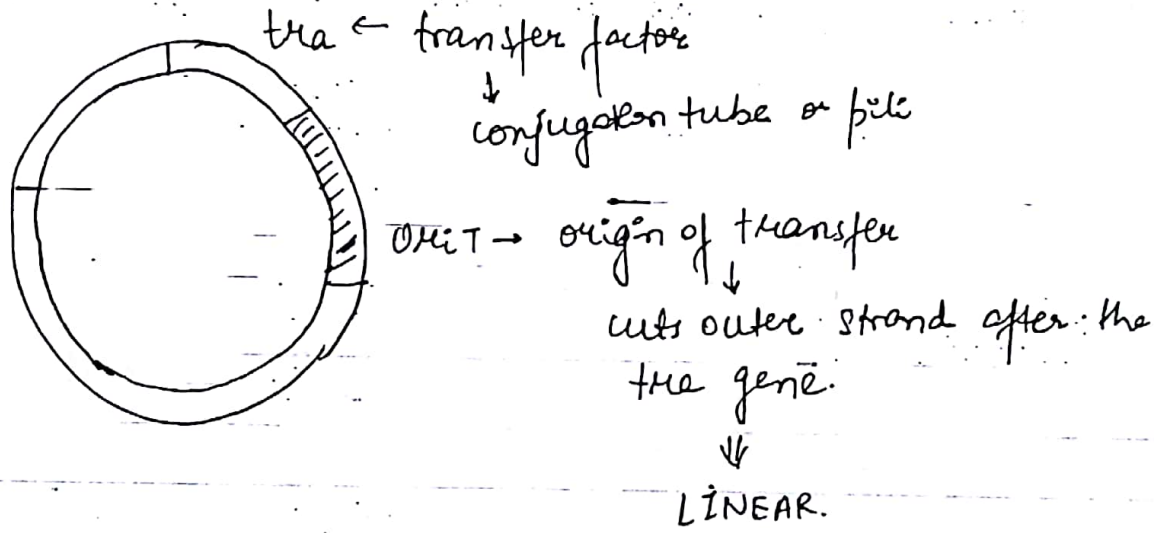
[ $F^-$  remains the same].

There are 4 genotype in bacteria

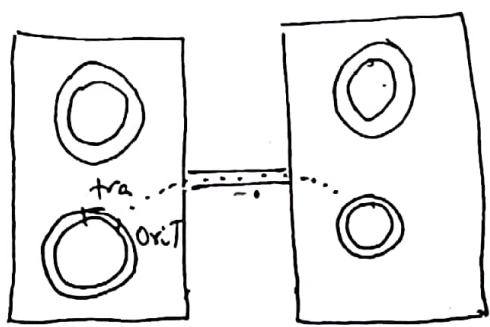


F plasmid

F' is the bacteria where plasmid contain chromosomal material



$F^+ \times F^-$



Plasmid is self replicating hence 1 strand from the other

- ⇒ - change in genotype.
- No Homologous recombination is not seen. Q.

↓  
No transfer of chromosomal genes

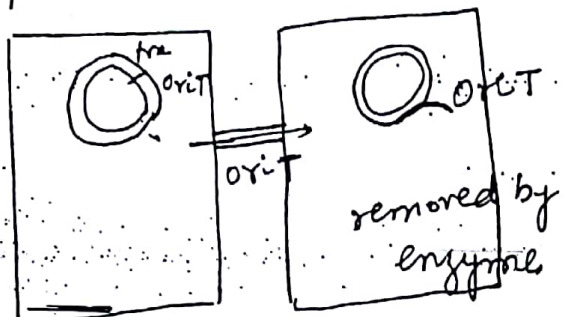
- Horizontal Transfer.
- Rapid

# Plasmid mediated Drug Resistance.

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$Hfr \times F^-$

conjugation tube is stable  $\approx 10 \text{ min}$



$\Rightarrow$  No change in genotype

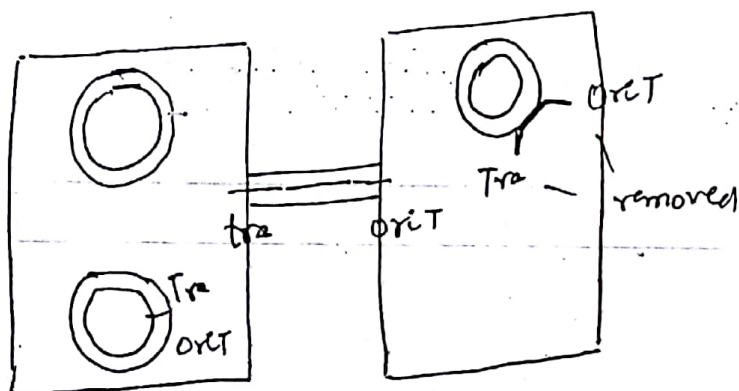
$\Rightarrow$  Homologous recombination is seen

$\Rightarrow$  Transfer of chr. genes

$\Rightarrow$  Horizontal  $\rightarrow$  not rapid

$F' \times F^-$

- Sexduction.



$\Rightarrow$  No change in genotype

- Homologous recombination is seen

- Transfer of chromosome genes

-  $\downarrow$  frequent

$F^+ \times F^+$   
 $Hfr \times F^+$

conjugation (X)

# STERILIZATION & DISINFECTION

Killing of all organisms including spores.

↓  
Biological Indicators (BI)  
↓  
10<sup>6</sup> ~~species~~ spores of *Bacillus* sp. } Quality control.

## STEAM STERILISER (AUTOCLAVE)

121°C for 15 min at 15 lb pressure

- Surgical equipment.

- dress material

- bandages

- culture media except L.J.

Loeffler's serum slope } ↓

By Inspiration

80°C for 1 hr x 3 days.

fractional  
sterilization.

## Tyndallisation

100°C for 1 hr x 3 days.

Sugar media → Heat at 100°C

BI → *B. stearothermophilus*

once a week.

**BEST**

Bowie Dick Test or Vacuum leak Test

chemical indicator

→ every run.

measure the penetration of steam.



## HOT AIR OVEN

160°C for 2 hrs.

→ glassware

→ Liquid

→ sharp equipments.

↳ autoclave (X)

→ chemical disinfectant can also be used for sharps

BI → B. Subtilis subspecies NIGER

↓

B. atrophaeus (new name)

## ETHYLENE OXIDE

Warm cycle → 50°C ± 5°C

Cold cycle → 37°C ± 5°C

{ plastic ware (syringe, IV tube, catheters, urine bag)  
gloves

Gamma waves are preferred.

ETO → Heart Lung Machine

BI - Bacillus Subtilis GLOBIGI

## IONISING RADIATIONS (cold sterilization)

$\gamma$  rays - flasks  
gloves  
catgut suture

BI  $\rightarrow$  B. pumilio

UV rays  $\rightarrow$  Biosafety cabinets

$\downarrow$   
HEPA filter Highly efficient particulate  
air ( $0.3 \mu\text{m}$ )

ULPA filter (ultra low particulate air  
( $0.12 \mu\text{m}$ ))

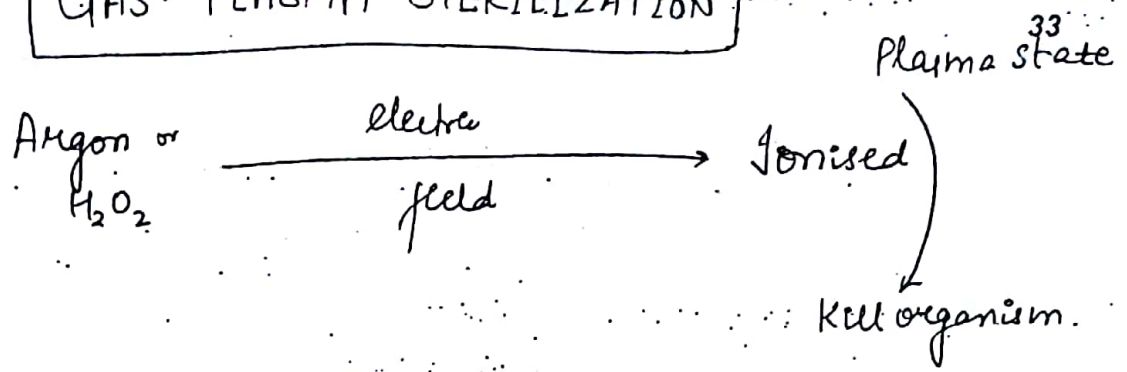
## FILTRATION

- Vaccines + anti-sera
- Membrane filters  $\rightarrow 0.22 \mu\text{m}$ .

BI  $\rightarrow$  Brevundimonas diminuta (pseudomonas)

Bubble point Testing.

# GAS PLASMA STERILIZATION



BI - B. atrophaeus

## DISINFECTION

Reduction of no. of pathogens including spores

## ANTISEPTIC

Chlorhexidine

## SPAULDING CLASSIFICATION

It classifies equipments

Critical  $\rightarrow$  tissue contact  $\rightarrow$  surgical equipments  $\rightarrow$  Sterilisation.

Semicritical  $\rightarrow$  mucous membrane  $\rightarrow$  Endoscopes / colonoscopes (heat sensitive)

$\downarrow$   
2% Glutaraldehyde for (20 min)  
(CIDEX)

Laparoscope (critical)  $\rightarrow$  2% Glutaraldehyde for (2 hrs)

Non-Critical → Skin → Thermometer

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↓  
intermediate level disinfectant

Ethanol

## CLASSIFICATION OF DISINFECTANTS

Level	<del>Level</del> Sporicidal	Virucidal Non-enve	enveloped	Others
High	+	+	+	+
Intermediate	—	+	+	+
Low	—	—	+	+

High → • 2% Glutaraldehyde

• formaldehyde

↓  
fumigation ←

Peracetic acid +  $H_2O_2$

Intermediate → alcohol

Phenol

$Cl_2$  releasing agent



Low → Quaternary ammonia  
Lysol (cresol + soap)

35

## EFFICACY OF DISINFECTANT

- 1) Concentration
- 2) pH
- 3) Contact time
- 4) Organic compound → ↓ efficacy except phenol

Sputum } 5% phenol. 18 hrs  
Stool }  
↓  
RNTCP

## TESTING OF EFFICACY

→ Phenol Co-efficient  
Reidel Walker  
Chick Martin (organic matter) } not used nowadays

→ In use Test (MIC)

↓  
→ Kelsey Sykes capacity test

↓  
In case of organic matter, this test can be done.

Endoscopes → 1st rinsed in  $H_2O$  to remove organic matter  
↓  
disinfectant

## Complication

→ M. chelonae abscess

PRIONS → autoclave at 134°C for 5 hrs.

or  
✓ 2N NaOH.

# IMMUNE SYSTEM

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Attribute	Innate	Adaptive
① Response Time	min/hours	Days.
② Specificity	Low (for str. shared by group of microbes)	High (for specific Ag of microbes. <sup>every epitope</sup> )
③ Diversity	Limited	High. $10^8$ to $10^{10}$ isotype
④ Memory	Low (only in NK cells)	High
⑤ Self/non self Discrimination	Yes	Yes
⑥ Anatomical physical barriers 1st barrier $\rightarrow$ low pH	Skin, mucosa, chemical (lysosome, IFN $\alpha$ & $\beta$ , temp. & pH)	L.N., spleen, MALT
⑦ Blood proteins	Complement	Antibodies
⑧ Cells	Phagocytes, monocytes, macrophages, neutrophils, NK cells, other leukocytes, epithelial, endothelial cells	Lymphocytes other than NK cells

Innate & adaptive Immune Response are interdependent & not independent.

## MATURATION OF CELLS

occur in 1° Lymphoid organs

↓  
Bone Marrow

~~Thymus~~

Antigenic exposure  $\rightarrow \boxed{2}$

↳ 2° Lymphoid organs

↓  
Spleen → blood borne

Spleen  
L.N. → Lymphatic draining tissue.

MALT (mucosa associated lymphoid tissue)

CALT (cut annotated " ")

## Intestinal pathogens

↓  
skin.

Bone marrow act as 2° lymphoid organs in few cases  
eg. Salmonella

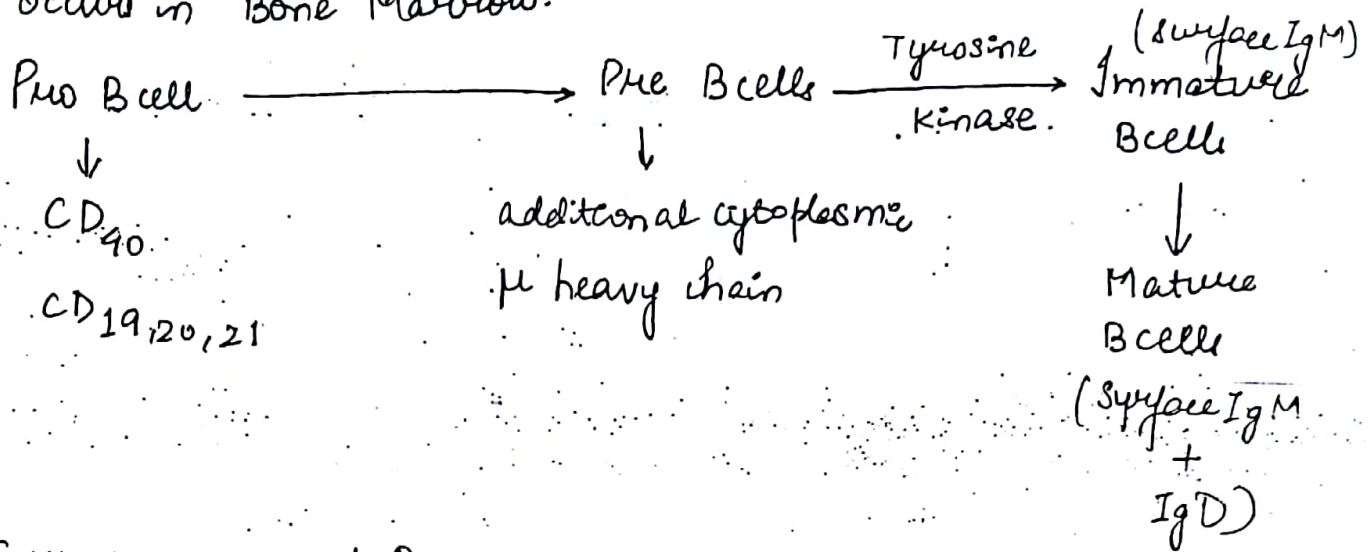
\* Organ : act as both 1° & 2° Lymphoid organ. Bone Marrow.



# B CELL MATURATION

39

occurs in Bone Marrow.



Follicular cell of Bone marrow

↳ carries out +ve feedback selection, -ve feedback selection.

- NO MHC

- They check the maturation ⇒ +ve selection.

sent to 2° lymphoid organs.

Few B cells

↓  
autoAb.

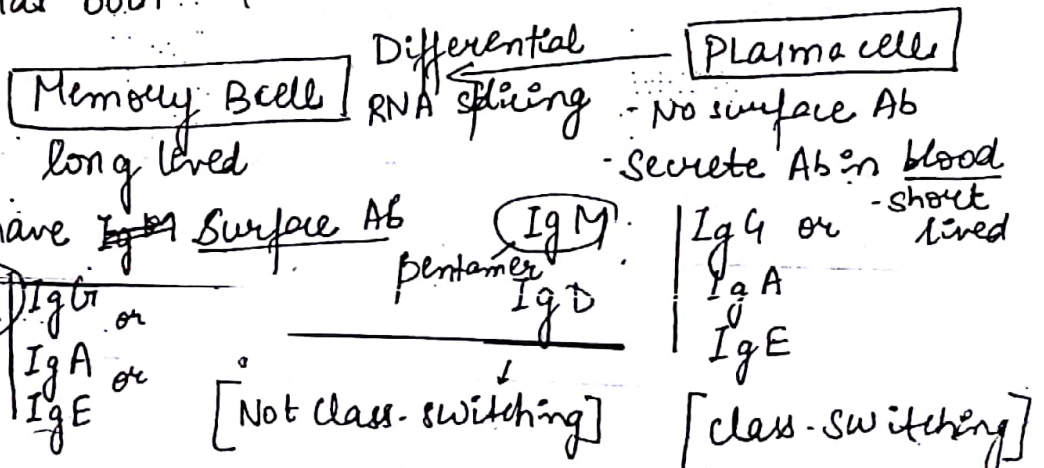
- Killed by apoptosis

↓  
-ve selection

prevents autoimmunity

antigen exposure

Hence B cell, has both +ve & -ve selection.



40

Q. One B cell  $\rightarrow$  1.5 to 3 lakhs surface Ig  
(mature)  
 $\rightarrow$  IgM & IgD  
1:1000

Peak Response Time in 1° Immune Response = 7-10 days  
Secondary " " " " = 3-4 days

- Occur in Bone Marrow & Thymus

cells expressing  
CD<sup>7-7</sup> go to  
thymus

Bone Marrow

Bcell  $\leftarrow$  CD

○ Lymphoid cells.

Notch 1 gene

CD7<sup>+</sup>

$$CD_1^+, CD_2^+, CD_3^+, CD_7^+, TCR^+, CD_4^+, CD_8^+$$

**Cortex**

Positive selection

TLR

Cell

Self MHC recognition.

$$CD_3^+ CD_2^+ CD_3^+ CD_7^+ TR^+ \leftarrow CD_4^+ PD_8^- :TH$$

Medulla

95% → Negative selection → few T cells have TCR & ↑ affinity for self MHC. ↓

Killed by apoptosis

2° Lymphoid organs

prevents  
autoimmunity

APC → MHC II & MHC I (nucleated cells)

41

Professional [Dendritic cells] → specialised



→ Macrophages

→ B cell

Non-Professional

→ Microglial cell (brain)

→ fibroblast on skin

→ pancreatic  $\beta$  cells

→ vascular endothelial cells

→ epithelial cells — N cells

Thymic epithelial etc.



All nucleated cells or platelets have MHC I

MHC-I → has 1 chain

MHC-II → has 2 chains

If affinity of T cell is quite more towards self MHC

↓  
It may act as autoantigen

↓  
Hence undergoes apoptosis

occurs in medulla

# LYMPHOCYTIC HOMING

42

Thymus Dependent  
area

Thymus Independent  
area

Spleen Periaarteriolar Lymphoid  
Sheath

Cortical medullary  
follicular

Lymph  
node Paracortical area

germinal centre

T cell zone

B cell zone

In a person undergoing thymectomy <sup>(neonates)</sup> at adolescence / LN Biopsy

↓  
Paracortical area undergoes atrophy  
↓  
as T cells are not formed

## TOLL LIKE RECEPTORS

TLR1 → Mycobacteria &  
Gram -ve bacteria

TLR2 → Gram +ve bacteria  
Trypanosome  
Mycobacteria  
Yeast & other fungi  
Schistosomes

TLR3 → Viruses



TLR4 → Gram -ve bacteria  
RSV  
Fungi

43

TLR5 → Bacteria

TLR6 → Mycobacteria  
Gram +ve bacteria  
Yeast & other fungi

TLR7 → Virus

TLR8 → Virus

TLR9 → Bacterial DNA  
Herpes virus

Malaria parasite heme byproduct

TLR10 → Unknown

TLR11 → Uropathogenic bacteria  
Toxoplasma

TLR12 → Unknown

TLR13 → Vesicular stomatitis virus

# ANTIGENS

44

Epitopes are found in antigen

↓  
They are separately immunogenic

Antigenic Determinants

1) Size < 10k Dalton → Hapten

10-100k Dalton → Hapten or Immunogen

> 100k Dalton → Immunogen

All antigens are not immunogens but all immunogens are antigens.

## 2) Chemical Nature

Protein > polysaccharide > Lipid > Nucleic Acid

## 3) Susceptibility to tissue enzymes

## 4) Foreignness

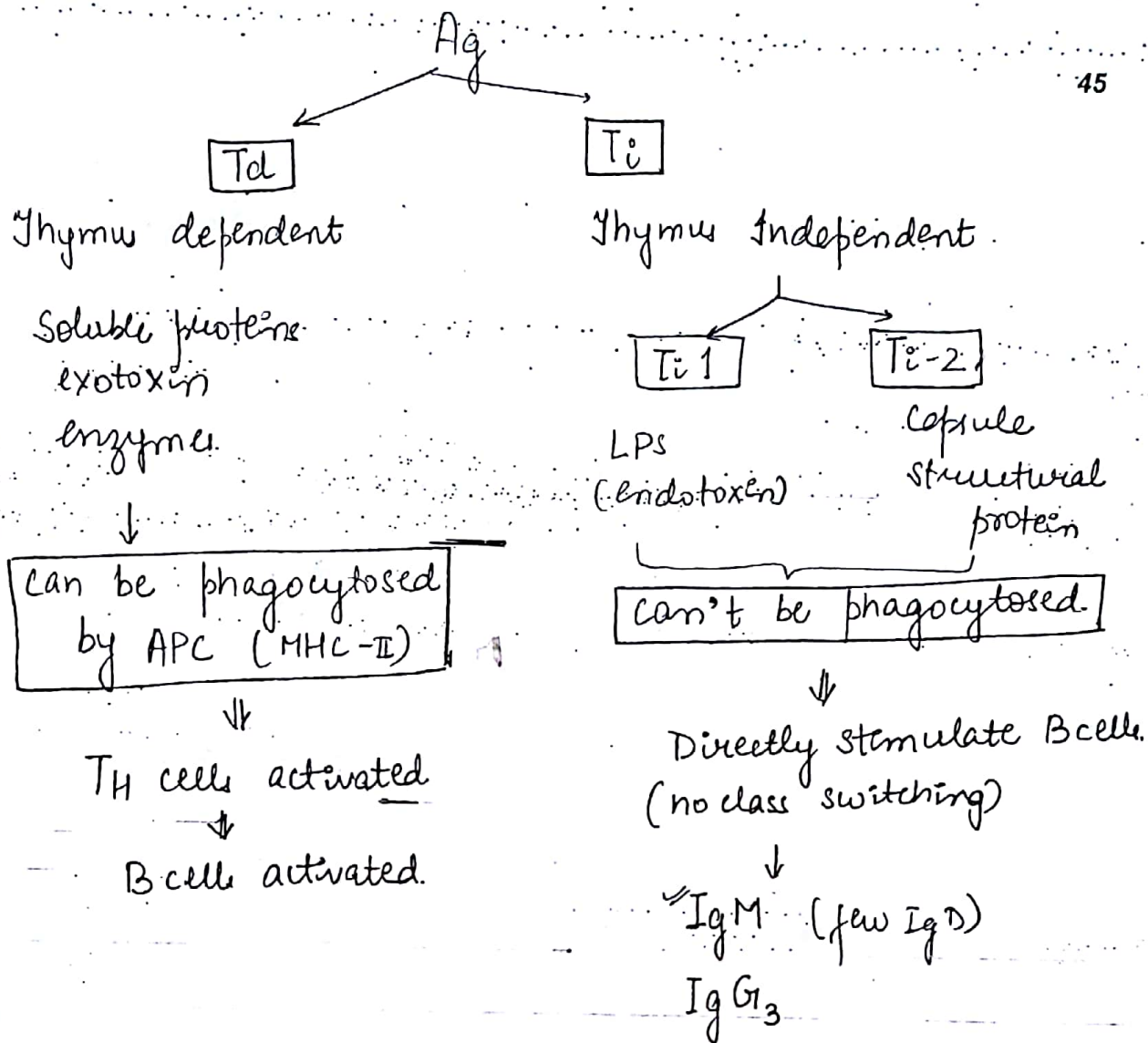
directly proportional

Sequestered Ag → lens protein  
↓  
sperms

can lead to  
autoimmunity

## 5) Immuno privileged sites

Cornea  
Uterus  
testis  
Brain } No immune response

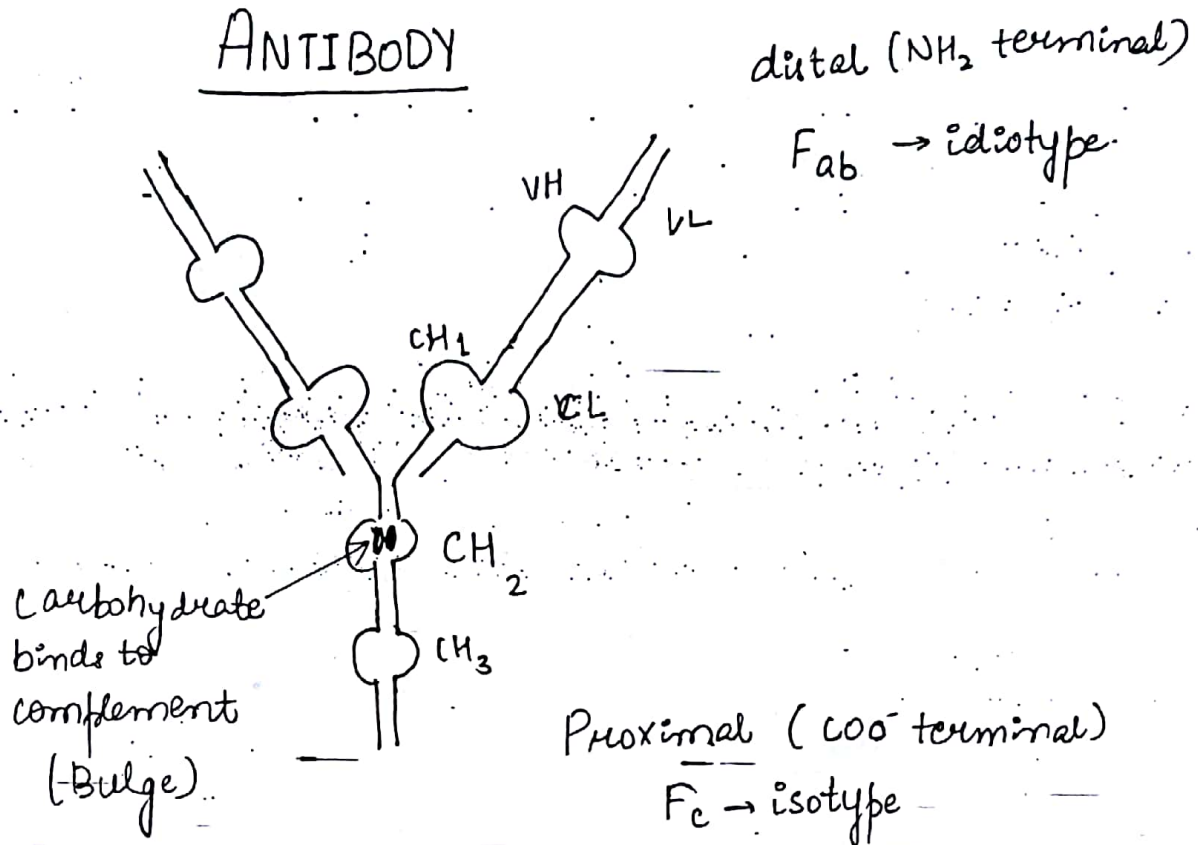


	Td	Ti
Class switching	(+)	(-)
Ab diversity	(+)	(-)
Affinity maturation	(+)	(-)
Memory response	(+)	(-)
Phagocytosis	(+)	(-)
Complement activation	(-)	(+)

B cell func<sup>n</sup> on cytokines released by T cells.

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## ANTIBODY



IgM, IgE → additional CH<sub>4</sub> domain.  
Carbohydrate → CH<sub>3</sub>

Molecular wt.

IgM → 900 kDa (millionaire)

IgA → 365 "

IgE → 190

IgD → 180

IgG → 150

Max carbohydrate content → IgE - 12%  
IgD - 13%



\* Idiotyp is determined by variable domain of both H & L chain. 47

Hyper variable region

[Complementarity Determining Region]

Each Domain has 3 CDR



3 Domain CDR

Paratope is the portion of Ab where Ag comes & bind.  
2 similar Ags can bind to both side.

Func<sup>n</sup> of Idiotyp - Antigen Binding.

\* Isotype is determined by constant region of mainly  
(H chain) & L chain

Func<sup>n</sup>  $\Rightarrow$  IgM  $\rightarrow$  complement activation

IgG  $\rightarrow$  opsonisation & ADCC (antibody mediated dependent cell mediated cytotoxicity)

IgE  $\rightarrow$  degranulation of mast cells  
basophils, eosinophils

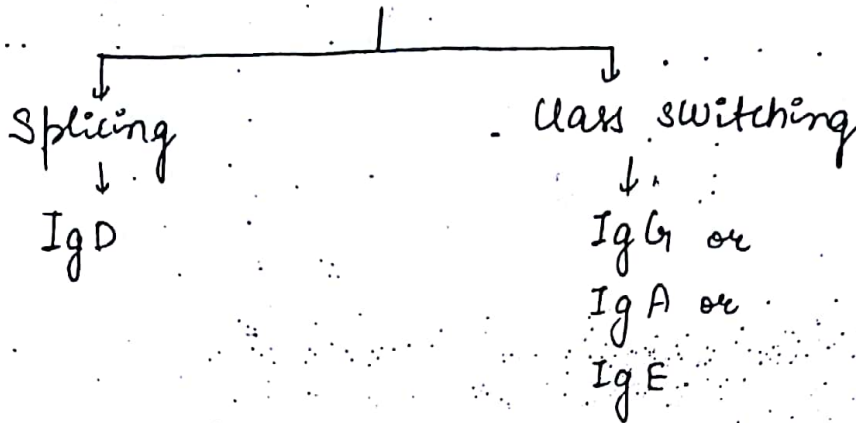
IgA  $\rightarrow$  destroy cell membrane of helminths

IgA  $\rightarrow$  mucosal immunity

IgD → no biological func<sup>n</sup>

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## MECHANISM OF ISOTYPE FORMATION :



Genes encoding

H chain

L chain (kappa)

(lambda)

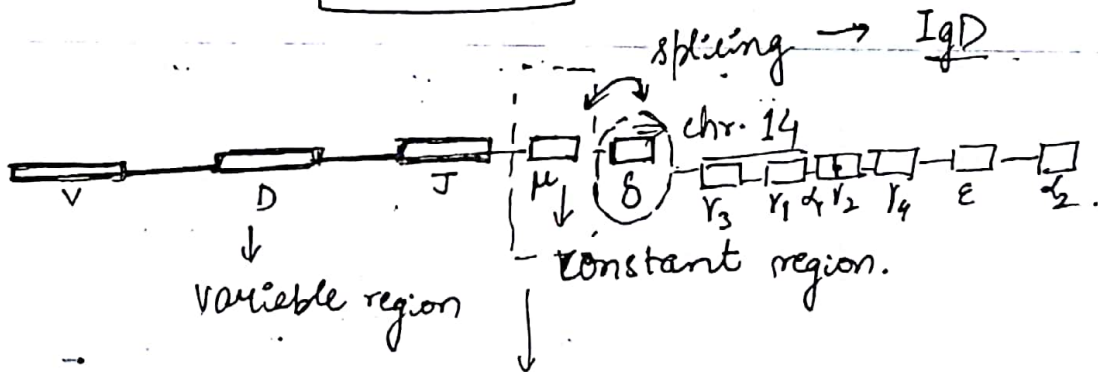
Located on:

— chr 14

— chr 2

— chr 22

$$K:\lambda = 2:1$$

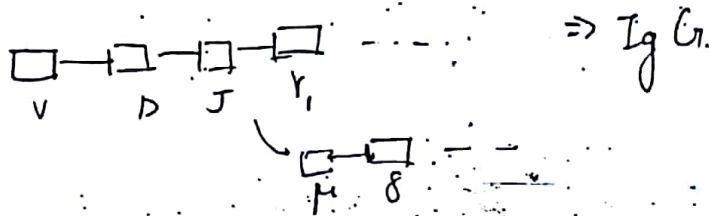
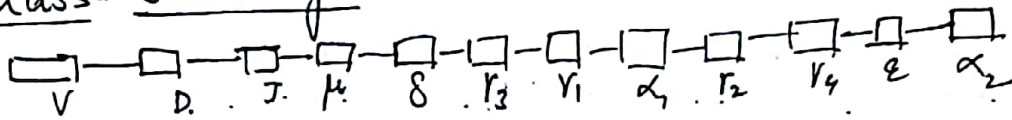


- Hence IgM is the 1st Ab formed
  - Marker of acute infect.
  - Whenever B cell stimulated.
- IgM is always formed

Class-switching occurs in B cell only once.

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### Class-Switching:



1) enzymatic removal of isotype determining gene —  
↓  
gene rearrangement

2) irreversible

3) occurs only once in one B cell

4)  $(T_H2) (CD_{40L}) \rightleftharpoons (CD_{40}) B cell$   
↓  
B cell get activated.

5) Germinal centre of 2° lymphoid organs.

## Nude Mice [natural strain]

- No hair
- Chr 11 defect on Fox N1 gene
- vestigial thymus  
↓  
selective T cell deficient
- Gnotobiotic environment [Germ free environment]
- IgM (few IgD) → No class switching

## Di George Syndrome

Fish Mouth

Deformed ears

Wide set eyes

Cyanosis ← cong. Heart Disease. [Fallot's H/c]

Seizure due to hypocalcaemia [no parathyroid]

No thymus

Defect - Chr 22 q 11 defect

- failure of development of 3<sup>rd</sup>, 4<sup>th</sup> pharyngeal pouch

no class switching → only IgM Ab formed



## \* Hyper IgM Syndrome

defect in CD40L on T cell

51

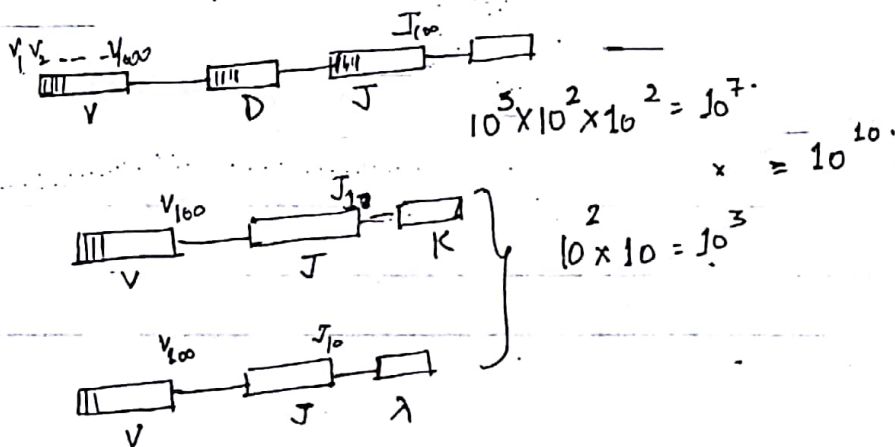
↓  
Selective B cell Deficiency

Classified on the basis of clinical outcome

- No class switching
- $\uparrow\uparrow \text{IgM} \rightarrow 10 \mu\text{g/mL}$  [N  $1.5 \mu\text{g/mL}$ ]

## Ab DIVERSITY

Mechanism of idiotype formation



M/c. Helminthic infxn in HIV = Strongyloides.

- Recombination of multiple genes encoding for variable domain of both H & L chains

↓  
Recombination of every H chain  
= all different L chains

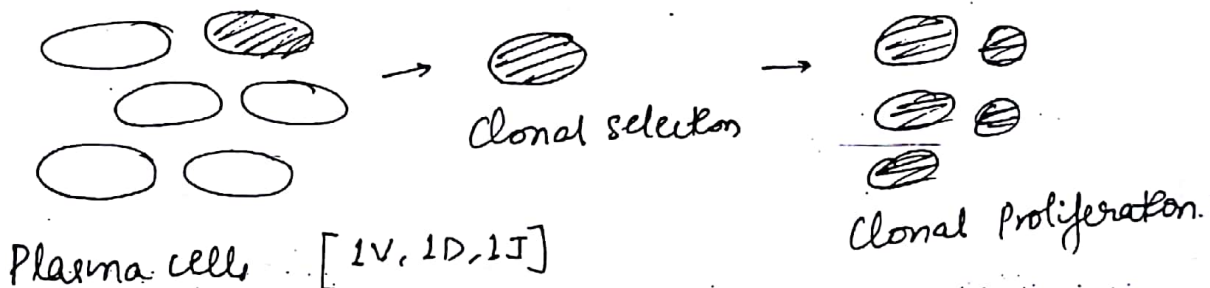
Nucleotides are getting added continuously, hence genes never get exhausted 52

Mutation also leads to addition of genes

1> Terminal nucleotide addition by tdt enzyme

2> Removal of nucleotide by exonuclease

## AFFINITY MATURATION (Somatic Hypermutation)



Mutation in the gene in such a way that Ag binds with greater affinity in Domains.

By mutation in the variable gene,

↓  
↑ affinity

↓  
Clonal selection of that cell  
rest cell dies

↓  
This cell undergoes clonal  
Proliferation

Through affinity maturation we can know about  
present or recurrent infection

past infect.  $\rightarrow$  high affinity  
present "  $\rightarrow$  low affinity

53

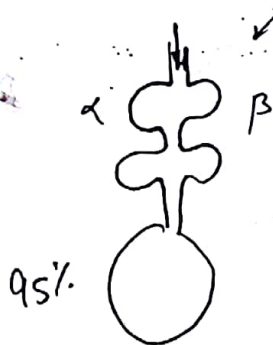
C Ab has  $\uparrow$  affinity?

IgG  $\rightarrow$  since time taken is more

IgM  $\rightarrow$   $\downarrow$  affinity  $\rightarrow$  but Highest ability (valency) 10

Valency of B cell Receptor = 2

T cell Receptor = 1.



18/2/18

M/c Mechanism responsible for idiotypes or Ab diversity  
= Recombination at the variable regions.

Affinity Maturation is due to mutation at the variable region genes.

C ~~Isotype~~ isotype has highest affinity IgG

C " " lower " IgM.

A pt. suffering from CID not susceptible to c Shigella species → Shigella dysenteriae.

The B cell stage predominant in Bruton hypogammaglobulinemia → Pre B cell

The cytokine responsible for pathogenesis of salmonella gastroenteritis → IL8

Negative selective helps in prevention of c type of disorders - Autoimmune

## ALLOTYPES

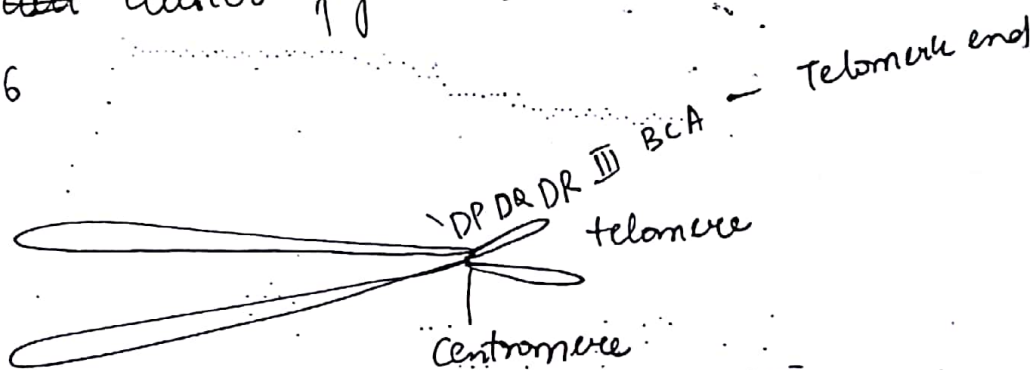
Subtle amino acid difference in the constant region of H chain & L chain.



## Allelic exclusion

## MHC [MAJOR HISTOCOMPATIBILITY COMPLEX]

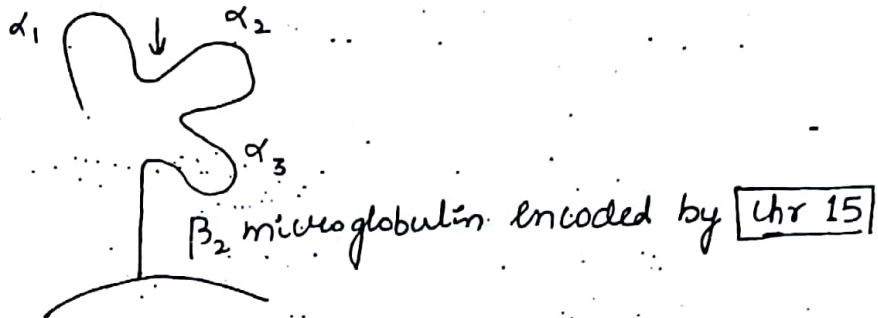
It is a ~~cell~~ cluster of genes located on short arm of Chr. 6



CD Player  
Centromere



## MHC-I



- Site of binding of Aq in MHC-I  $\Rightarrow$  Distal  $\alpha_1$  &  $\alpha_2$ .
- Peptide of length 8-10 AA can bind to this site
- Deficiency of  $\beta_2$  microglobulin leads to
  - Hereditary Haemochromatosis
  - Due to mut<sup>n</sup> in 283 position of Hfe gene



Due to this mutation

Cysteine is replaced by Tyrosine

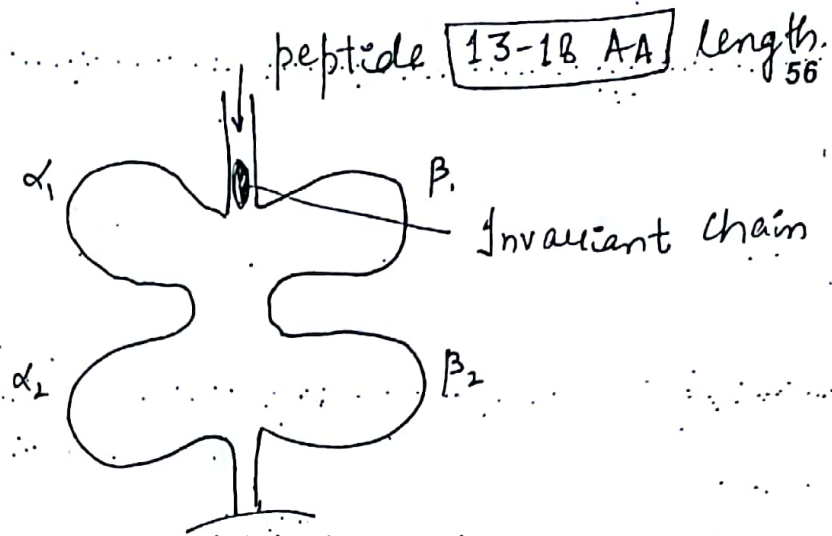


[ $\beta_2$  microglobulin regulates absorp<sup>n</sup> of Fe]



So Iron overload may occur

## MHC-II



- Invariant chain prevents blocks binding of normal peptide.

↓  
Hence MHC-II prevents autoimmunity

- Defect in Invariant chain leads to autoimmunity  
[is due to defect in DQ, DR region]

## MHC-III

It codes for complement protein  $C_4$  +  $C_2$   
Heat shock proteins

TNF  $\alpha$  +  $\beta$

21 Hydroxylase.

# ANTIGEN PROCESSING (Td)

57

Td Antigens

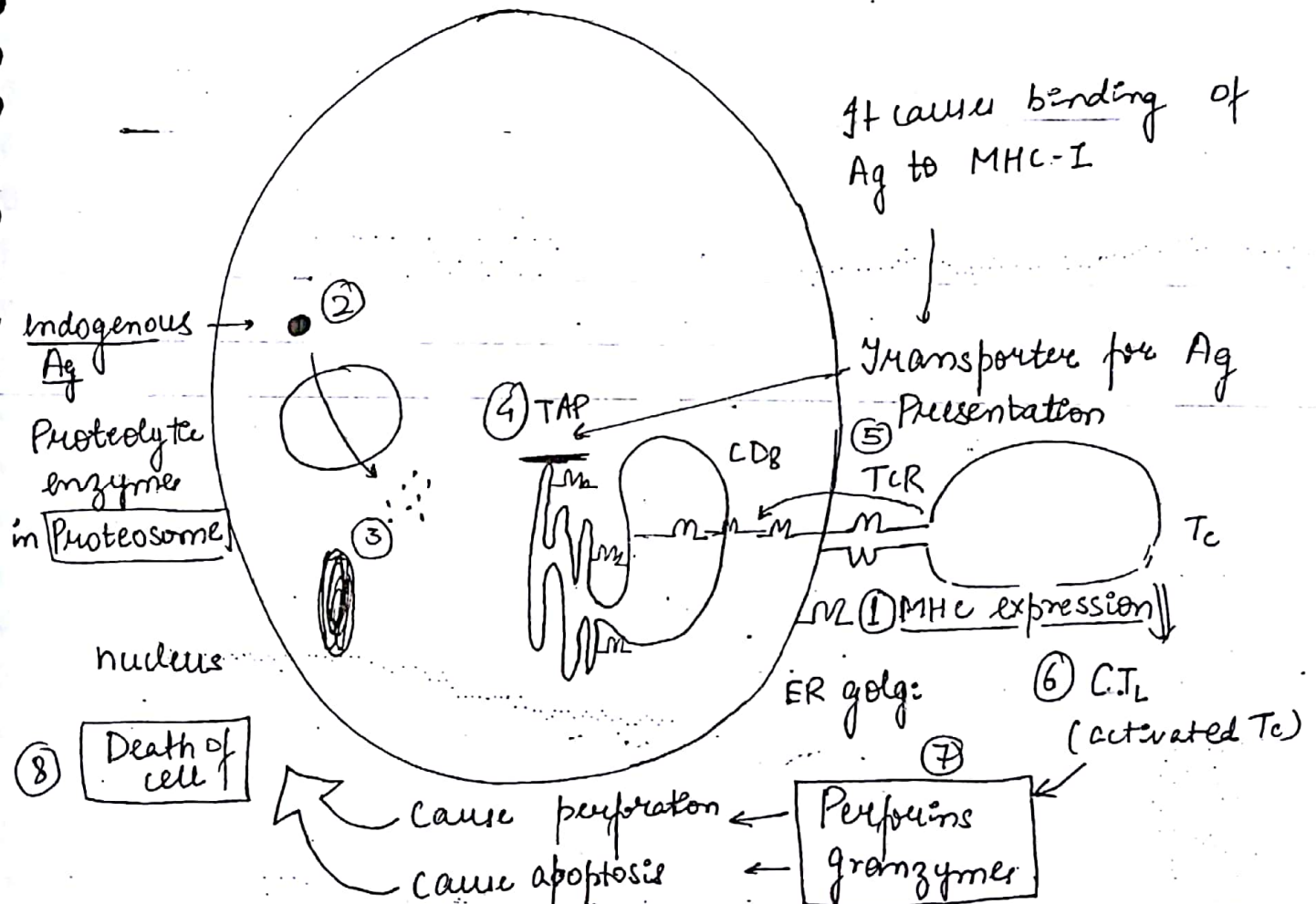
Endogenous Ag

Exogenous Ag

Tumour Ag cells

Virus infected cells

## I Endogenous Ag Processing



Proteolytic enzymes break endogenous Ag into smaller proteins

Tc (CD8) binds to  $\alpha_2$  domain of MHC I [Tc]

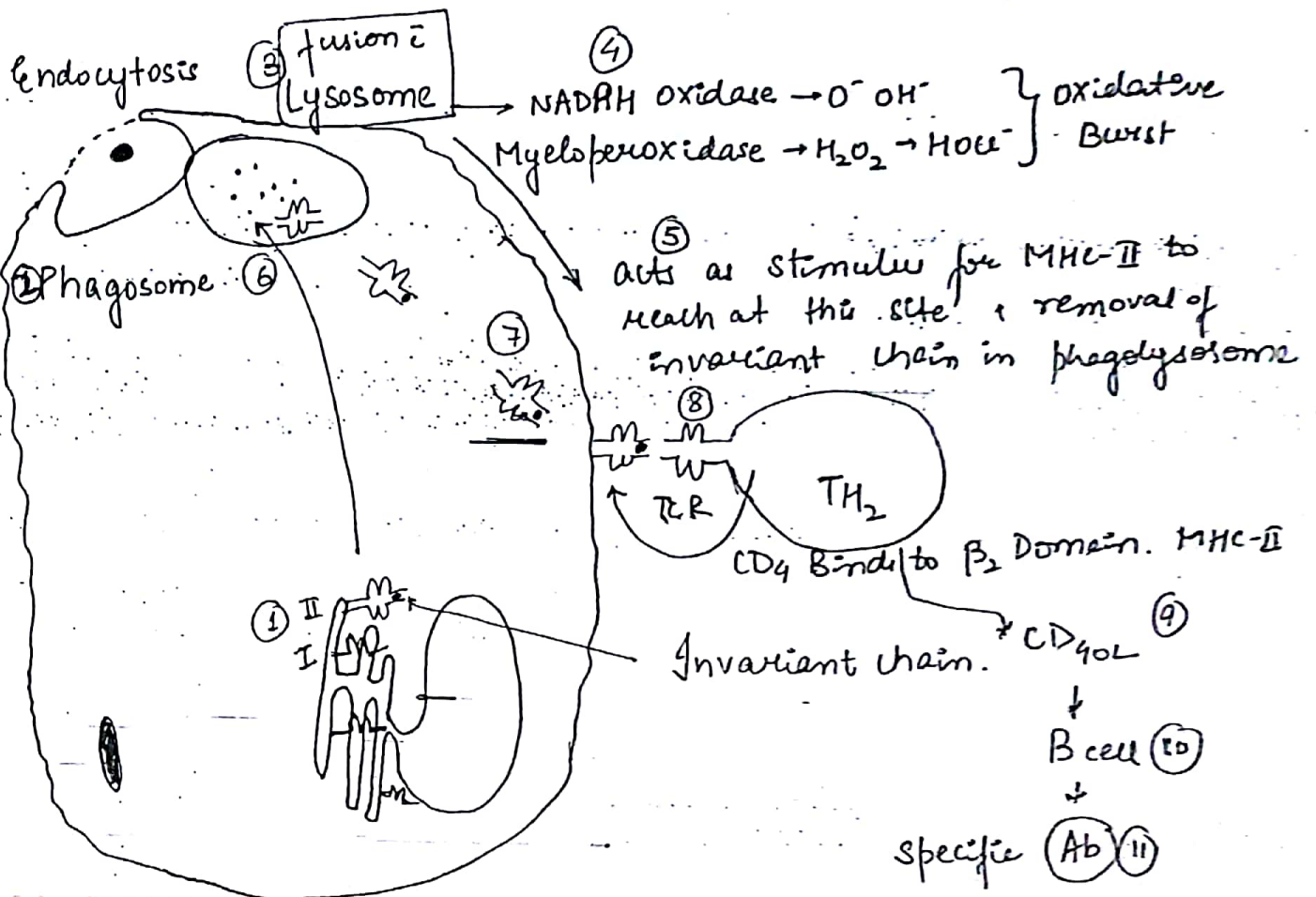
CD4 binds to  $\beta$  chain \*





# \* Exogenous Ag. Processing

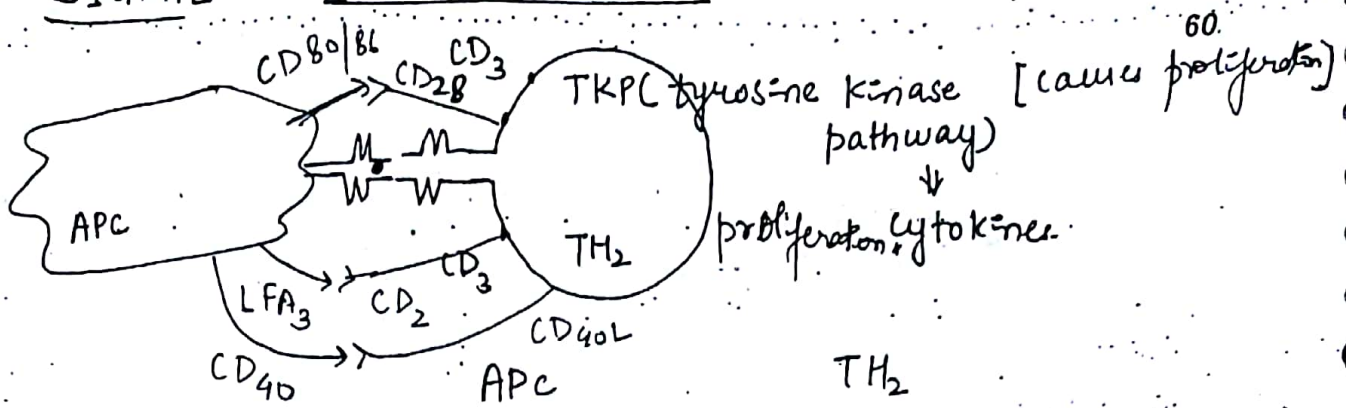
B cell  $\rightarrow$  pinocytosis 59



## SIGNAL TRANSDUCTION OF TCELL

# SIGNAL

# TRANSDUCTION



1st Signal

Ag restricted  $\bar{c}$   $\longleftrightarrow$  TCR  
MHC II

2nd Signal

(costimulatory  
Signal)

$B_7$  (CD80/86)  $\longleftrightarrow$  CD28

LFA3  $\longleftrightarrow$  CD2

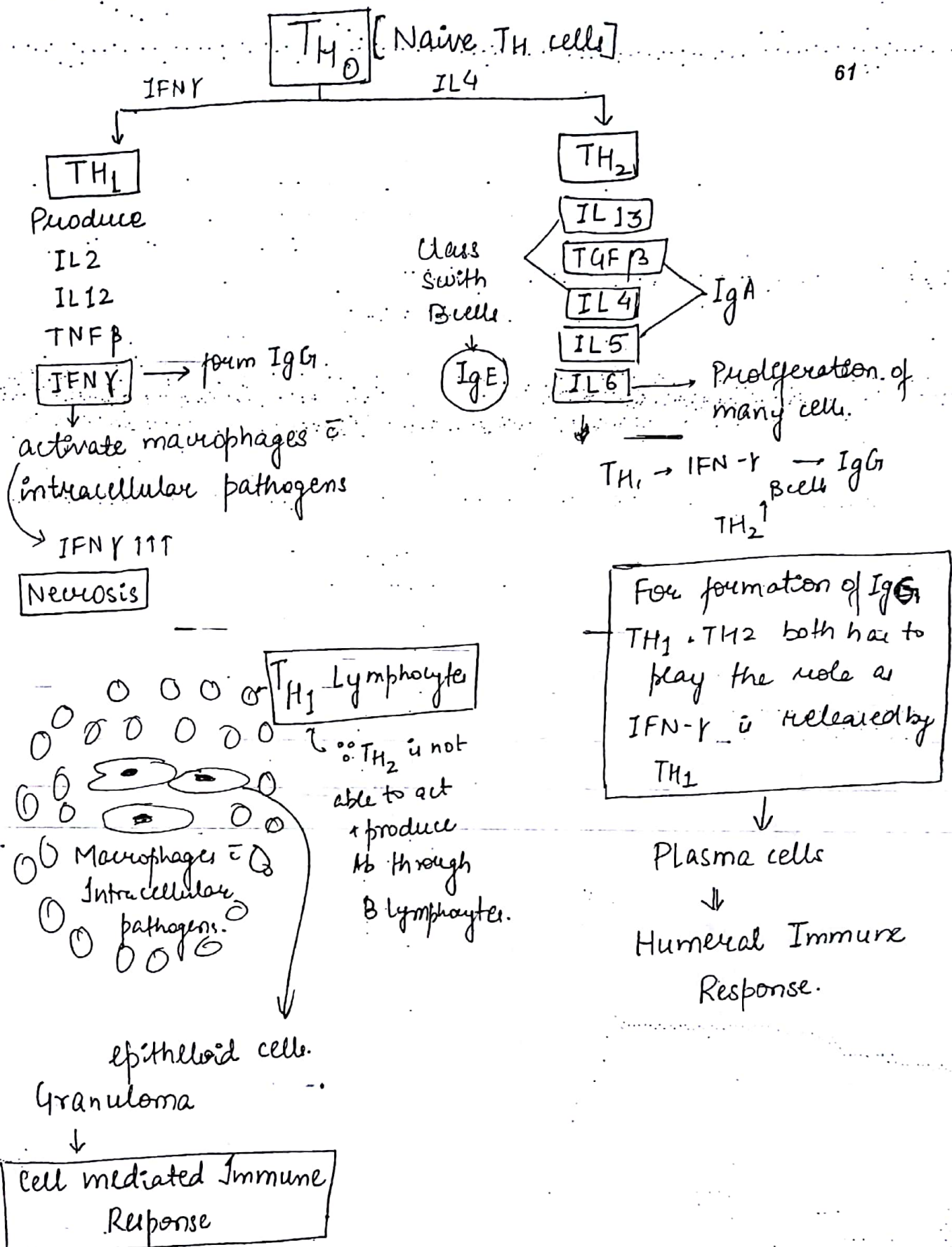
CD40  $\longleftrightarrow$  CD40L

3rd Signal

Signal Transduction

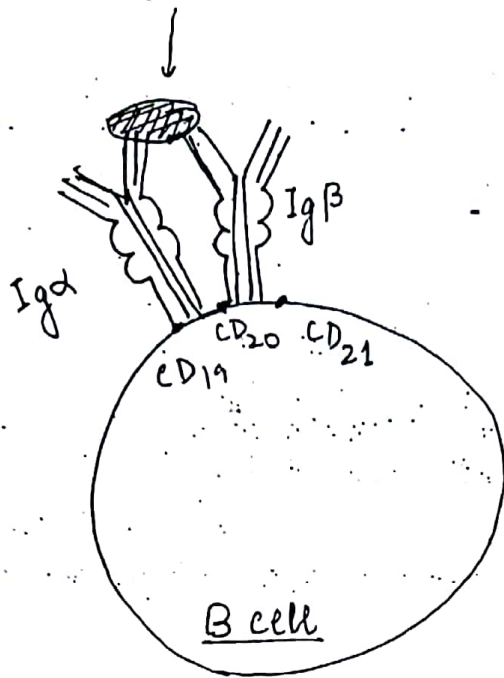
CD3 activated

$\downarrow$   
TKP activated  
 $\downarrow$   
Proliferation  
cytokines  $\uparrow\uparrow$



Ti Ag

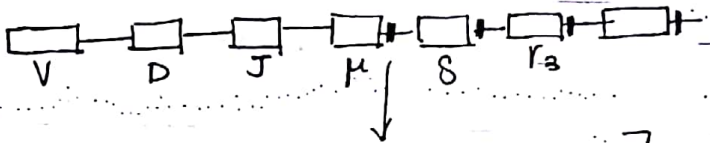
62



Ig Superfamily

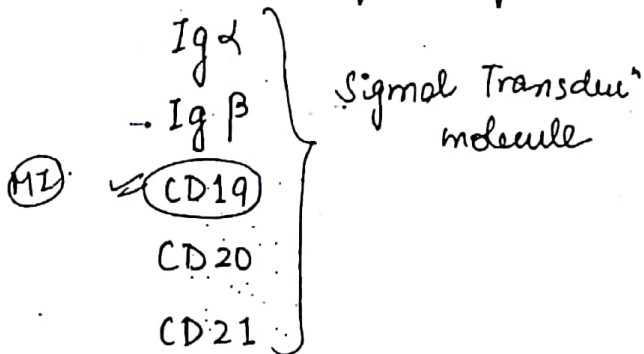
$Ig\alpha + Ig\beta$

H + L chain



This gene encodes for  $Ig\alpha \cdot Ig\beta$  chains } Differential RNA Splicing (Plasma  $\rightarrow$  Memory)

Signal Transduction by Ti Ag



Rest is same [TKP activation + following]

$IgM \rightarrow$  Complement System



# COMPLEMENT SYSTEM

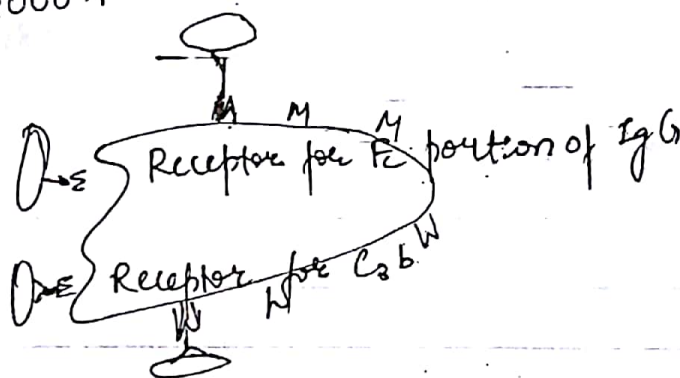
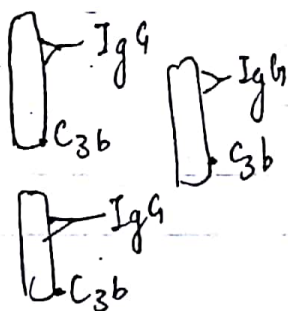
63

## Functions :-

- 1) Opsonisation ( $C_{3b}$ )
- 2) MAC mediated lysis of bacteria ( $C_{5b-9}$ )
- 3) Immune complex clearance ( $C_{3b}$ )
- 4) Chemotaxis by smaller fragments ( $C_{5a}$ )
- 5) Ag-Ab complex activation
- 6) Memory

## OPSONISATION

↑ phagocytosis  $\Rightarrow 4000 \times$



## Opsonins

### Innate response

Collectins SPA,

SP-D,

MBL

L-ficolin

C1q

\* C3b

C4b

C3b

cleavage products ( $iC_{3b}$ ,  $C3c$ ,  $C3dg$ )

### Adaptive response

IgA

IgG\*

CRP

Max. complement protein  $\Rightarrow$  Bound  $C_3$ .

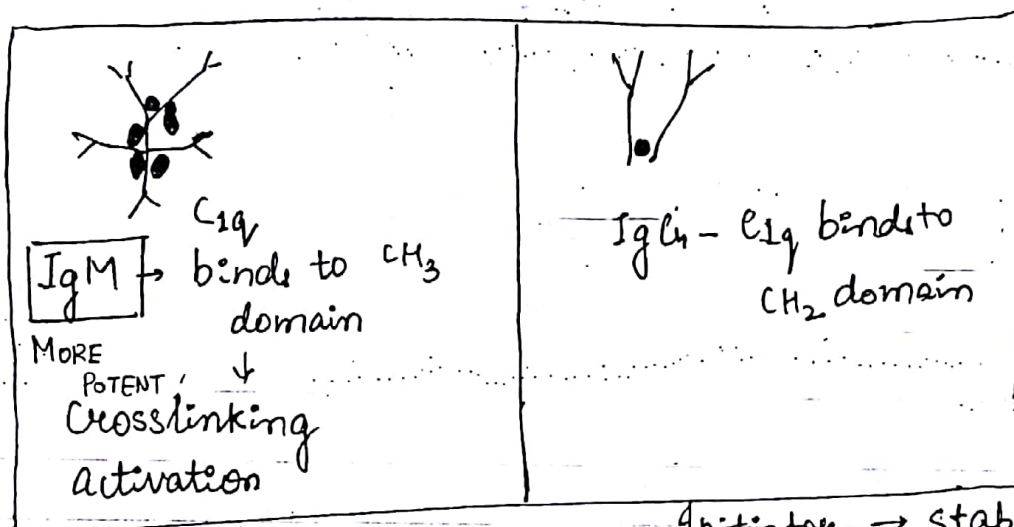
64

$C_3$  converter bound  $C_3 \leftarrow \begin{matrix} C_{3a} \\ C_{3b} \end{matrix}$

(I) **Classical**  
IgM / IgG

(II) **Alternate**

Free  $C_3$  in blood  $\leftarrow \begin{matrix} C_{3a} \\ C_{3b} \end{matrix} \rightarrow$  degrade



IgM / IgG binds to Ag +  $C_{1q}$

$C_4 \rightarrow C_{4a}$   
 $C_{4b}$

Initiator  $\rightarrow$  stabilize  $C_{3b}$

Factor D or Properdin

Factor B

Ba  $\rightarrow$  Bb

$C_{4b2a}$   $C_3$  convertase  $\rightarrow$  bound  $C_3$

$C_{3bBb}$   $C_3$  convertase

$C_{3a}$   $C_{3b}$

$C_5$  convertase

$C_{3bBb3b}$   $C_5$  convertase

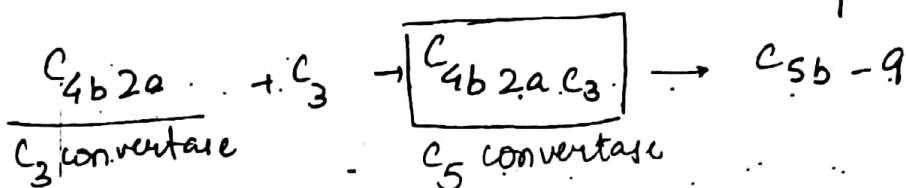
$C_{5b-9}$

Neisseria Inf<sup>n</sup> ← Lysis  
It doesn't allow opsonisation.

MAC

65

So,



### Indicators of Alternate

- 1) Endotoxin
- 2) Snake venom
- 3) Yeppanosoma protein
- 4) Zymosan on yeast
- 5) Nephritic factor
- 6) Dextran sulphate
- 7) Inulin
- 8) IgG, IgA, IgD.

$C_{4b}$  binds to  $C_2$ , expose it to the action of  $C_{1s}$

↓  
 $C_{1s}$  cleave  $C_2$  into  $C_{2a}, C_{2b}$

Ne  
Yes Snake, In & End Ymy, Kia  
→ GRAD

IgE, IgG<sub>4</sub> doesn't activate complement

### (11) LECTIN PATHWAY

MBL (mannose binding lectin)

↓  
It activates  $C_{1q}$  directly

↓  
rest similar to classical pathway.

# REGULATION OF COMPLEMENT

66

- | <u>Protein</u>                             | <u>Function</u>   |
|--|---|
| 1) CI Inhibitor                            | → Dissociation of inhibition of $C_{1r}$ & $C_{1s}$ from $C_{1q}$ |
| 2) Decay accelerating factor (CD55)        | → Dissociation of $C_3$ convertase                                |
| 3) <del>CR1</del><br>C4BP<br>Factor H      | } → Dissociation of $C_3$ convertase<br>Cofactor for factor I     |
| 4) Factor I                                |   |
| 5) Membrane cofactor of proteolysis<br>MCP | → Cofactor for factor I   |
| 6) S protein or vitronectin                | → Binds soluble $C_5b_6_7$ & prevents insertion into host         |
| 7) Protectin (CD59)                        | → Blocks binding of $C_9$ & formation of MAC                      |
| Carboxypeptidase N, B & R                  | → Inactivates the anaphylatoxins $C_{3a}$ & $C_{5a}$              |



Deficiency of  $C_3$   $\Rightarrow$  No Opsonisation.  
(bound)  $\downarrow$

Pyogenic Infect.  
GN

67

Deficiency of Late complement proteins  $\Rightarrow$  Recurrent Neisserial  $\downarrow$  Infection.

[MAC mediated Lysis  $\ominus$ ]

Deficiency of Decay accelerating factor (CD55)  $\Rightarrow$  PNH.

Deficiency of  $C_1$  inhibitor = Hereditary Angioneurotic edema

$\downarrow$

Swollen lips —  
Painful abdominal edema  
Laryngeal edema

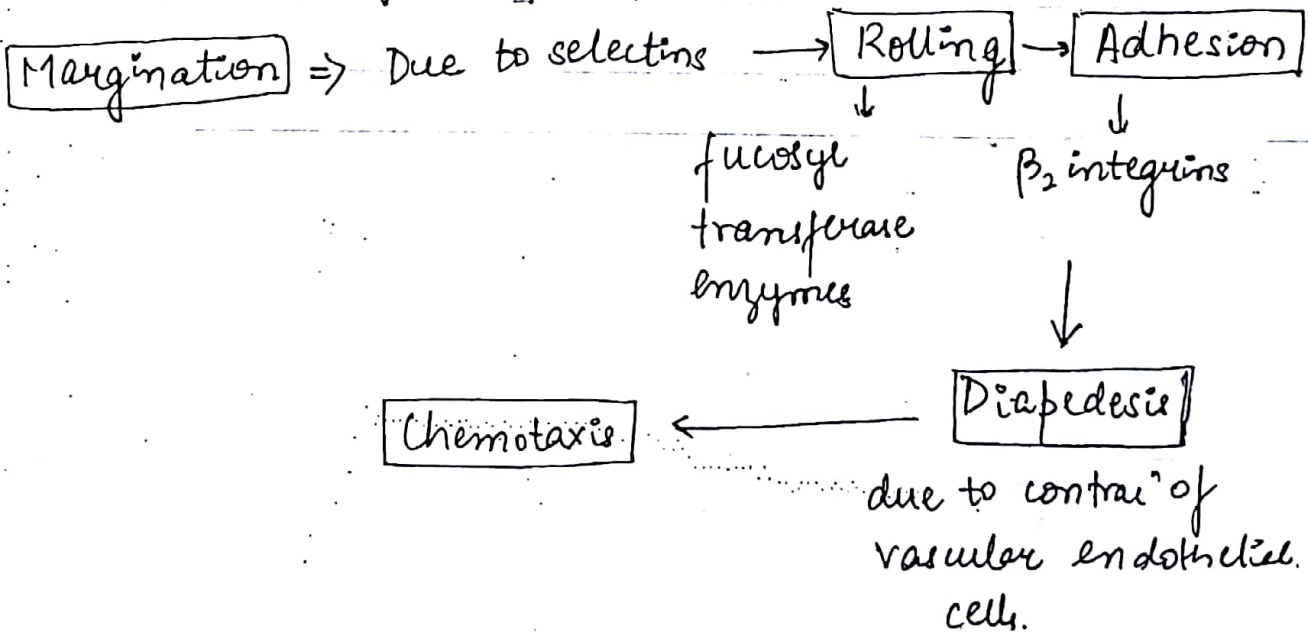
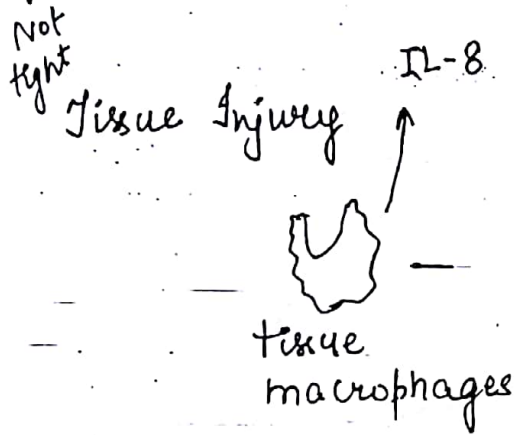
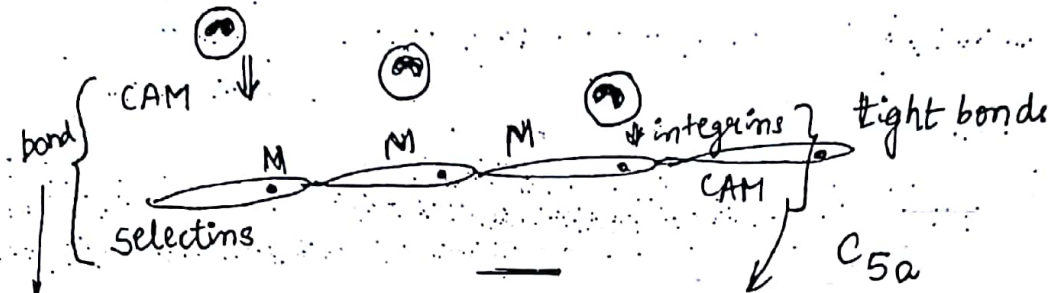
$\Delta$  - Best marker

$\downarrow$   
 $\downarrow C_4$  Level

# PHAGOCYTOSIS

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## Extravasation of Neutrophils

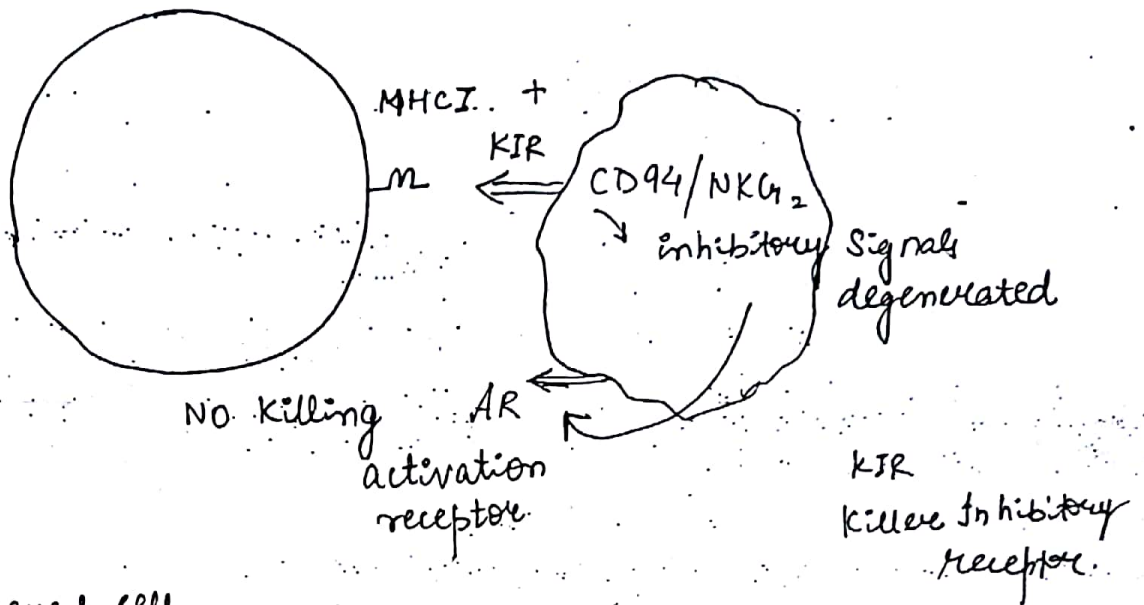


# NK Cell

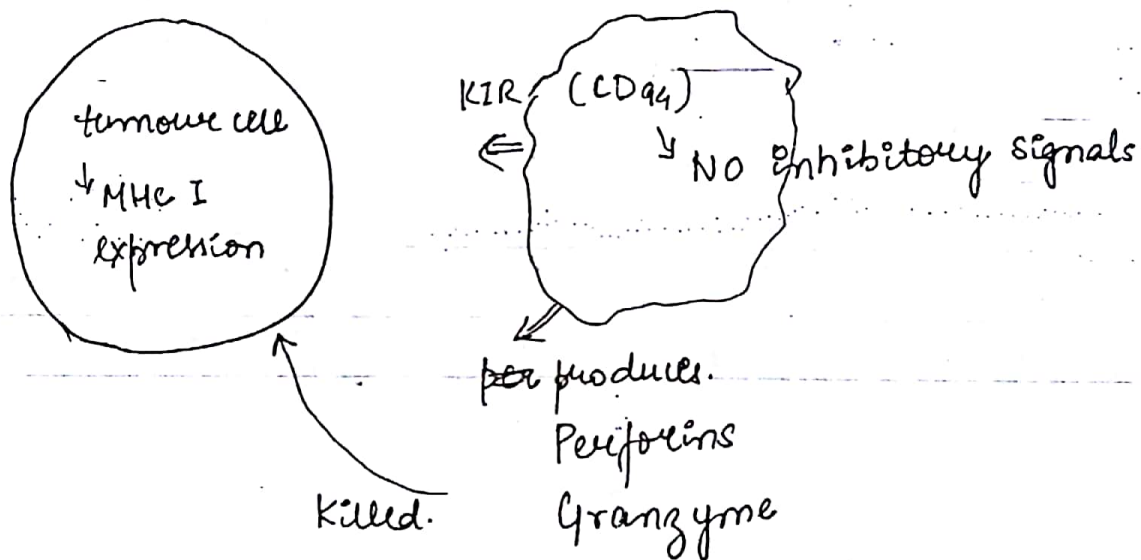
MHC-I in exogenous Ag

69

Normal cell

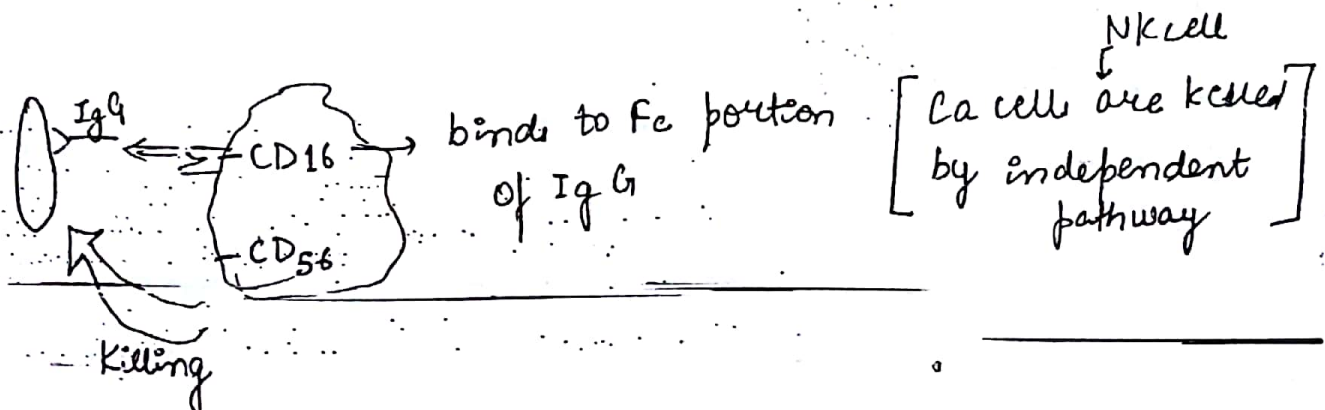


altered cell



Ab independent killing by NK cells.

NK cells → ADCC (Ab dependent cell mediated cytotoxicity)



# DEFICIENCY OF PHAGOCYTOSIS

70

## (I) Leucocyte Adhesion Deficiency (LAD)

Recurrent infections

No pus  $\rightarrow$  as neutrophils can't come out

Omphalitis

Extreme neutrophilia ( $>30,000/\mu L$ )

**LAD I**

$\rightarrow$

mutation in  **$\beta_2$  integrin** (CD18), gene (adhesion)

AR

**LAD II**

$\rightarrow$

Deficiency of **fucosyl transferase** [selectin]  
 $\downarrow$   
rolling  $\ominus$

**LAD III**

$\rightarrow$

Deficiency Defect in **regulatory protein Kindlin**  
(fermt3)  $\subseteq$  activates ligand for  $\beta_2$  integrin.

**LAD I > LAD III > LAD II**

## (II) G6PD Deficiency

Deficiency of enzyme in **HMP shunt**

Same as CGD (chr 4q-10)  $\bar{c}$  associated

Anaemia



### III. MPO Deficiency Granule enzyme Deficiency

71.

### IV. Chediak-Higashi Syndrome

AR

Defective Intracellular transport Protein (LYST).  
Recurrent infect<sup>n</sup>, Chemotactic factor, degranulation defect.

~~Absent~~ NK cells

Partial albinism.

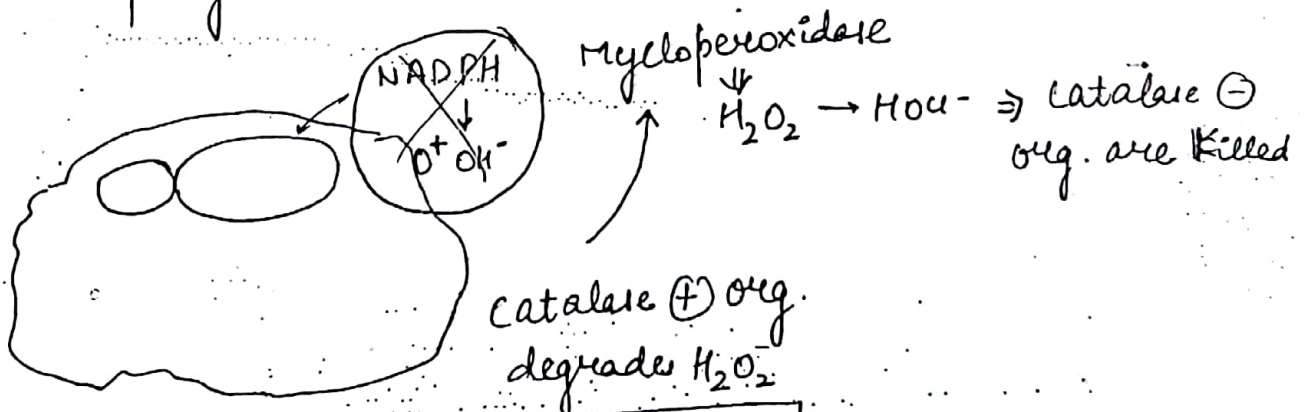
### $\Delta$ - Giant Lysosome

### V. Chr. Gr. Ds

- XL or AR

- Deficiency of NADPH.

- Recurrent Infection of  $\bar{c}$  Catalase +ve Bacteria  
& Fungi



Candida	Staph. aureus
Aspergillus	Haemophilus Inf <sup>n</sup>
	Mtb
	Enterobacteriaceae

① NBT (Nitroblue Tetrazolium) ⊖

2) Neutrophil Oxidative Index

if agammaglobinemia  
recurrent catalase +ve infec<sup>n</sup> ] ⇒ Bruton's  
NBT ⊕

B cell Deficiency

1) Bruton agammaglobinemia

Deficiency of tyrosine kinase B cell maturation  
XL

No Ig.

Prec B cells in bone marrow  
narrow CMI

2) X Linked hyper IgM Syndrome

- Deficiency of CD40L on activated T cell

- Recurrent resp. + GI infec<sup>n</sup> [IgA is absent]  
↳ mucosal immunity

3) Selective IgA deficiency

IgA - M/c deficiency

Repeated sinopulmonary + GI infec<sup>n</sup>

No role of passive immunisation



Passive Immunisation  
Delivers ⇒ IgG

#### 4) Common Variable Hypo $\gamma$ globulinemia

Onset - Late teens

B cell  $\uparrow$  in blood

$\downarrow$  Ig over time

$\uparrow$  autoimmunity

73

#### 5) Transient $\gamma$ hypogammaglobulinemia of infancy

Delayed onset of normal IgG synthesis

Detected in 5<sup>th</sup> - 6<sup>th</sup> month.

Resolves by 2 1/2 years

#### 6) Job Syndrome [Hyper IgE syndrome]

Due to deficiency of T helper 17 [TH17] cells.

Retained 1<sup>o</sup> tooth

Cold abscess

Coarse facies

Eczema

TH17 cell  $\rightarrow$  encoded by Chromosome 17

Differentiated from TH0 cell by IL-6, TGF $\beta$ , IL-23

TH2 produces  $\rightarrow$  IL-17, IL-22

IL17A IL17F

$\downarrow$

Stimulate B cells to produce

$\uparrow$  Ab except IgE

$\downarrow$

① Prevent pyogenic fungal Inf

② Inflammation

③ autoimmunity

④ transplant rejection. (ADCC) 74

p40 knock out Mice  $\rightarrow$  Deficient  $T_H17$

$\downarrow$   
 $\uparrow \uparrow$  IgE

## T cell Deficiency

1> Di George Syndrome

2> Bare Lymphocyte Syndrome (BLS) [Image]

Granulomatous necklesing Lesion in mid face

Type I  $\rightarrow$  TAP Deficiency  $\rightarrow$  MHC I  $\downarrow$

Type II  $\rightarrow$  MHC II  $\downarrow$

## Combined partial T & B cell Deficiency

Wiskott Aldrich Syndrome

XL

Defect in cytoskeletal glycoprotein (WASP)

Ataxia Telangiectasia

Defect in Kinase involved in cell cycle

Ataxia

Telangiectasia

Def of IgA & IgE



# Complete Functional B & T cell Deficiency 75

## SCID

- IL-2R $\gamma$  or JAK 3 deficiency

[cytokine Receptor deficiency]

Defective signals from IL4, 7, 9, 15, 21  
XL, AR

- Adenosine Deaminase ADA or PNP [Purine nucleoside phosphorylase deficiency] toxic metabolite in T, B cells.  
AR

- Zeta chain associated protein ZAP deficiency  
defective signal from TCR

- Rag 1 or Rag 2 nonsense mutation, AR  
No TCR or Ig gene rearrangement  
Total absence of B & T cells

# HYPERSENSITIVITY

76

	Immune mediator	Immune Response	Response Time
Type I	IgE	Humoral	2-30 min
Type II	IgG > IgM	"	5-8 hr.
Type III	Immune complexes (IgG)	"	2-8 hr
Type IV	T cells	Cell Mediated	24-72 hrs.

## TYPE-I

→ 1° response to allergen



Sensitisation of mast cell

ε IgE



→ 2° response to same allergen



Cross-linking

↓  
degranulation



## Mediators of Type I HSN

1°

Histamine, Heparin

Serotonin

Eosinophil chemotactic factor

Neutrophil chemotactic factor

Proteases (tryptase, chymase)

2°

77

PAF

Leukotrienes (SRS-A)

PGI<sub>2</sub>

Bradykinin

Cytokines

IL 1, 3, 4, 5, 6, 10, 13

TNF $\alpha$

TGF $\beta$

GM-CSF

Eg.

1) Anaphylaxis

2) Atopy

3) Allergic rhinitis (Hay Fever)

4) Asthma

5) Food allergy

6) Allergic eczema

7) ABPA (Type I > Type III > Type IV)

[allergic bronchopulmonary aspergillosis]

## Type - II

78

Autoimmune

Cytotoxic

Non-cytotoxic



- ADCC

↑  
Complement mediated  
autolysis

cellular func<sup>n</sup> altered by  
autoantibody.

eg. Grave's Ds

eg.

1) Autoimmune haemolytic  
anaemia

2) Aggranulocytosis, thrombocytopenia

3) ARF

4) Goodpasture Syndrome

5) Transfusion Reac<sup>n</sup>  
(ABO incompatibility)

6) Erythroblastosis fetalis  
(Rh incompatibility)

7) Drug induced haemolytic  
anaemia

[black water fever]  
[penicillin therapy]

8) ~~Myocarditis in Chagas Ds~~  
(American Trypanosomiasis)

9) Hyperacute Graft Rejection

1) Myasthenia Gravis

2) Grave's Ds

3) Type II non-insulin dependent  
DM

4) Pernicious Anaemia



## Type - III

Non-specific

79

Immune complexes



causes tissue damage



extravasation of neutrophils



reach site of injury



focal area of granuloma

Eg.s

1) SLE

2) Rheumatoid Arthritis [III > IV]

3) Polyarteritis Nodosa

4) Multiple Sclerosis

5) Serum Sickness

6) Arthus Reac<sup>n</sup>

7) PSGN

8) Lepus Reac<sup>n</sup> Type II

9) Meningitis

10) SAGE

11) Hepatitis B + C (arthritis)

12) Mononucleosis

13) Dengue (arthritis)

14) 5<sup>th</sup> disease (step chick)

15) Nephrotic Syndrome in P. malariae

16) Katayama fever in  
schistosomiasis

17) African trypanosomiasis

18) Penicillin +

Sulphonamide allergy

## Type IV

80

### Intracellular Pathogens

- 1) M. Leprae, M.Tb
- 2) Listeria monocytogenes
- 3) Brucella abortus
- 4) Pneumocystis jirovecii
- 5) Candida albicans
- 6) Histoplasma capsulatum
- 7) Cryptococcus neoformans
- 8) Herpes simplex virus
- 9) Variola
- 10) Measles

### Skin Test

Tuberculin, Lepidomin

Montenegro, Fice Test

Contact Dermatitis

Pyruyl chloride

Hair Dye

Nickel Salts

Poison

Ivy

Poison Oak

Hashimoto's thyroiditis

Type I Insulin Dependent  
DM

Guillain Barre

Cellar disease

Graft Rejection [IV > II]

Lepre React Type I.

Hypersensitivity pneumonia  
[IV > III]

# BACTERIOLOGY

81

## Drug Resistance

### Chromosomal

Mech

→ Mutation in the chromosome  
eg. Mtb

→ Transduction  
eg. MRSA

Con

### Plasmid

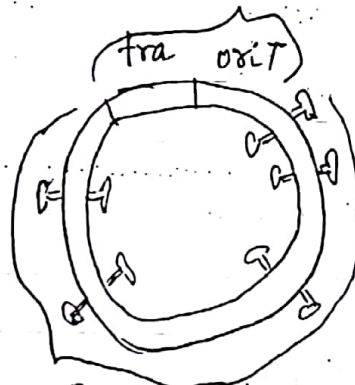
Mech

→ Insertion of Transposons  
carrying blood resistance genes  
in plasmid



Antibiotic Selection Pressure

RTF = resistance transfer factor



Rd - resistance determinant

R plasmid = Rd + RTF

Combination = useful  
Rx

Metabolic Defects



eg. INH (R) Mtb →  
catalase ⊖  
peroxidase ⊖

Not useful

> 8 drug (R) seen.

not seen.

(Plasmid doesn't code for  
metabolic)

# Anti-microbial Susceptibility Testing

82

M<sup>c</sup> Muller Hinton Agar

Middle Brook - M.tb.

Blood Agar - Haemophilus

Method

## 1) Dilution Method

Broth dilution

Agar dilution

## 2) Diffusion Method

More common

- STOKES meth [European]
- Kirbybauek  
(Disk Diffusion) [Imge]  
as per CLSI  
(Clinical Laboratory Std. Inst.)  
USA

## \* Broth Dilution method for MIC testing

Serial dilution of Ab

↑

Std. inoculum

Bacteria: 0.5 McFarland (M/C)

Fungi: 2 McFarland

↓

Density best measured by  
Spectrophotometer

Min. Bactericidal Conc<sup>n</sup> (MBC) ⇒ estimated by Subculture

$$\boxed{MBC \geq MIC}$$

MEC - Min. effective conc<sup>n</sup>

Done for Anti-fungal Susceptibility

Min. conc<sup>n</sup> at which distortion of Hyphae is seen  
morphological



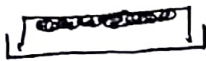
## Disc Diffusion Test

83

- CLSI
- 9cm diameter Petri Plate
- 6 disc used each 6mm diameter.



Incubate



Hazy → resistance  
clear zone → inhibition.

MIC can't be assessed.

- E test (epsilon test) [Image]

Diffusing Ab Gradient on a strip  
✓ Diffusion technique in  $\leq$  MIC can be tested.



← MIC

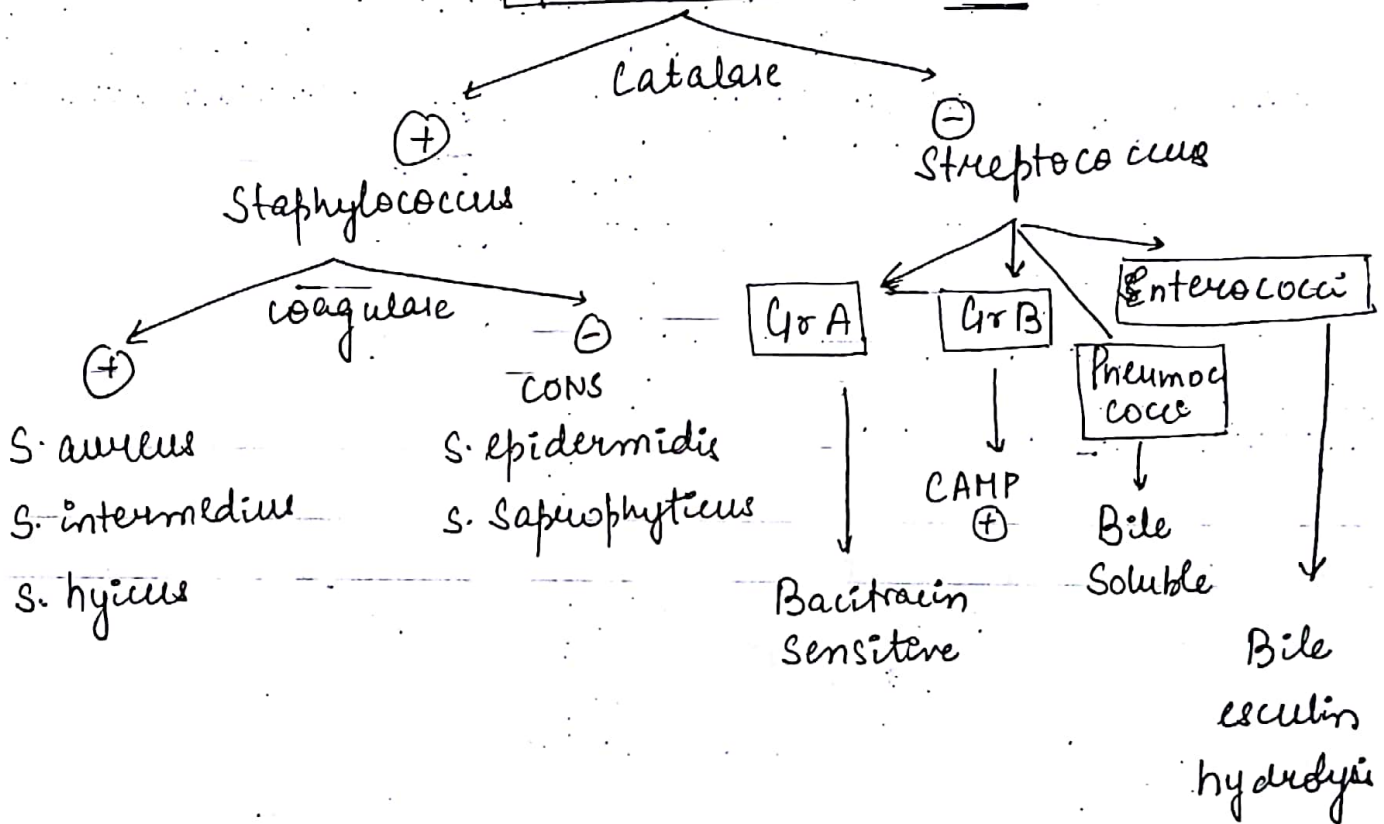
# GRAM +ve COCCI

84

Staph  
Catalase (+)  
Grape like clusters

Strepto  
(-)  
chains.  
S. salivarius - longest

## Gram +ve cocci



# STAPH. AUREUS

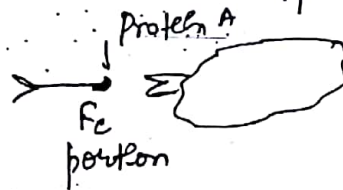
85

## I) Virulence Factors

↓ susceptibility to opsonisation

### 1) Protein A

- opsonin / Ab depletion by protein A
- binds to Fc portion of IgG. thus preventing binding of Ab to macrophages



- 90% of Staph have protein A [COWAN I strain]

### 2) SCIN (Staphylococcal complement Inhibitor protein)

binds + inactivates the  $C_3$  convertase of alternate pathway

### 3) Protease/

Degradation of IgG,  $C_3b$  by Staphylokinase

### 4) Clumping Factor activated Factor I.

### 5) Extracellular Fibrinogen Binding Lectin.

Inactivate  $C_3$  or prevents cleavage of  $C_3$ .

### 6) Capsule

prevents binding of phagocytes to opsonins

1) Peptidoglycan

2) Teichoic Acid

### 37. Toxins

a) Hemolysin - M/c of  $\beta$  haemolysis,  $\uparrow$  virulent  
 $\alpha, \beta, \gamma, \delta$   
 $\beta$  hemolysis

$\beta$  toxin  $\rightarrow$  sphingomyelinase  
 $\downarrow$  secreted  
 $\downarrow$  virulent

$\gamma$  toxin  $\rightarrow$  Pantone Valentine leucocidin [PVL gene]  
 2 component toxin [S & F]  
 $\rightarrow$   $\bar{c}$  binds  $\bar{c}$   $\gamma$  toxin

Synergohymenotrophic toxin

Q. associated  $\bar{c}$  Community Acq MRSA

b) Epidermolytic Toxin / Exfoliative Toxin -

Destroys mucopolysaccharide of Stratum Granulosum



BULLAE (coalesce)



SSSS [Staph scalded skin Syndrome] [Image]

children - Ritter's dx } fatal.  
 Adult - TEN

Pemphigus neonatorum } milder form  
 Bullous Impetigo }



$\Delta$  = toxin detection.

87

Enterotoxin =

SuperAg M/c  
cause TSS 1  $\rightarrow$  by B, C + F.  
8 types.  $\leftarrow$  Phage typing  $\rightarrow$  4cc I  
A B C<sub>1</sub> to 3 H I F.  $\downarrow$  Tampons.

$\Delta$  - Nikolsky Sign +ve.  
desquamation on pressure

Food Poisoning

— Type A M/c

Due to preformed Toxin

IP < 6hrs

vomiting (vagel nr stimulation)

'outbreak'

$\Delta$  - culture of vomitus / food.

47 Coagulase Enzyme.

Free.

Enzyme

activate coagulate reacting  
factor (CRF) in Rabbit / Human plasma  
 $\downarrow$  used in lab

fibrinogen  $\rightarrow$  Fibrin.

Tube Coagulase Test [Image]

Bound.

Clumping factor

Slide test

Tube coagulate

1:6 dilution plasma

Read at 4hr.

Released in log phase

88

5) Hyaluronidase ↑ virulent

It destroys tissues

II

M/c of boils / abscess

" osteomyelitis except

" Sickle cell anaemia → salmonella

" IV drug abuser → Pseudomonas

" epidural abscess

" native valve endocarditis

" Hospital acquired infection

III

Δ

Culture on 5-10% Blood Agar

Selective media → Ludlam media

Salt milk Agar

Pigment → Nutrient Agar

(Golden Yellow)

glycerol monoacetate

Specific Test for S. Aureus = Mannitol Fermentation Test (+)

Phosphatase (+)

Heat stable nuclease (+)

*S. intermedius*

ZOO NOTIC

→ Dog

89

Q In 2 wks, 5 newborns in the NICU developed *S aureus* bacteremia. PFGE of the isolates were similar. Which of the following should be done next.

- a) Prophylaxis of all newborns in the NICU w/ I/V vancomycin
- b) Protective isolation of all newborn
- c) Ensure strict hand hygiene
- d) Colln of nasal swab of health care workers.

Best Typing Method → **PFGE** (pulsed field gel electrophoresis) or sequence based typing.

**Phage Typing** [Image]

↳ Susceptibility to different phages among *S. aureus* strains

for virus = agarose gel electrophoresis preferred

Lysis out ⇒ If 1 bacteria infected w/ many phages.  
(undergoing any replication of phage)

Uses :- 1) *S. aureus*

**M/C phage Type in India = Group II**  
Most useful in epidemiology

2) *Shigella*

3) *Vibrio*

**M/C = *El Tor***

4) *Salmonella*

**M/C - *E<sub>1</sub>***

Least useful in epidemiologically



Vi phages used in salmonella

90

Vi Ag = S. Typhi  
S. Paratyphi  
S. Dublin  
Citrobacter

Father of Hand Hygiene = Edward Semmelweis  
obstetrician  
5<sup>th</sup> of May - Hand Hygiene Day

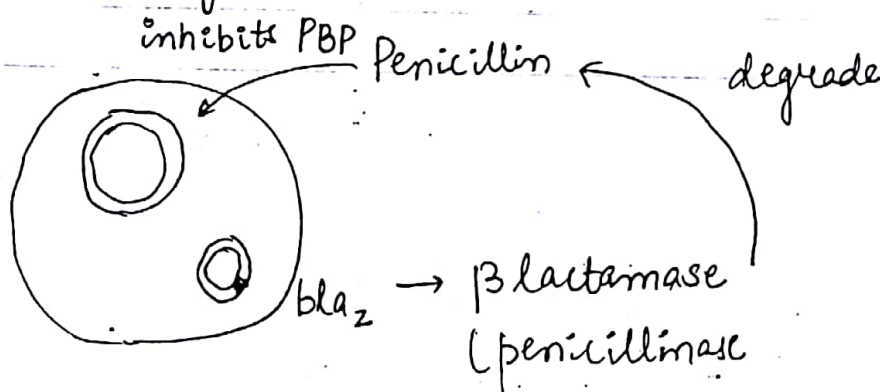
5 moments

Before & after seeing a pt

Before & after a procedure

Contact & pts. surrounding

#### IV Drug Resistance



- Plasmid encoded Drug (R) → By ~~Transformation~~
- By Transduction - 90%
- Conjugation - 10%

Penicillinase Resistant Penicillin  $\Rightarrow$   $\ominus$  PBP  $\rightarrow$  ~~degrade~~  
Penicillinase

~~Methicillin~~

~~Dicloxacillin~~

~~Oxacillin~~

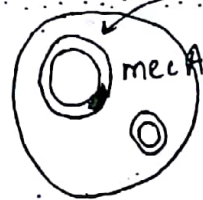
~~Carbenicillin~~

~~Cloxacillin~~

~~Nafcillin~~



## Transduction



*S. Scirei* (non pathogen) → grow at  $< 35^{\circ}\text{C}$ .  
91  
new PBP 2a  
(altered binding site)  
↓  
MRSA

## MRSA

Hospital Acquired Infection

Chromosomal

DOC → for t/tting MRSA → Vancomycin

Screening → Nasal swab (50% colonisation)  
Hand 40% colonisation

↓  
Mannitol Salt Agar + Cefoxitin

↓  
yellow colonies  
30°C → incubation [mecA gene expression  
best at 30°C]

TcBC media for Vibrio.  
Green coloured due to citrate

Q. 25 yr old girl presents to OPD with carbuncle at the back of neck. Pus aspirated reveals MRSA. Is it true about the strain.

a) ↑ Resistance

b) ↓ virulence

c) Associated with SCC mec I II III [Staphylococcal cassette chromosome → pathogenicity island]

dx ————— " ————— " ————— pvl

## Criteria of HAI :-

92

after 48 hrs of admission  
or

in 2 weeks of Discharge from hospital  
or

in 12 ~~weeks~~ months of Discharge in case of prosthetic implant

HA-MRSA

↓ virulent

SCC<sub>Hec</sub> I II III

↑ Resistance

↓

Clindamycin (R)

D test (+)

CA-MRSA

new strain

↑ virulent

SCC<sub>mec</sub> (IV) V VI

prl associated

↓ Resistance

↓

Clindamycin susceptible

(-)

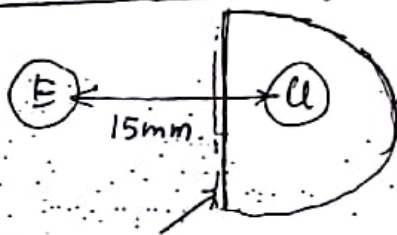
Q D Test

To detect Inducible Clindamycin Resistant Strains  
expressing ERM gene

↓

Erythromycin - induces Clindamycin (R)

This can't be done by Diffusion Test



← susceptible strain  
grows away as resistant  
is not developed this side

Resistant strains

due to erythromycin grows  
towards clindamycin

VRSA

Plasmid encoded

VanA gene derived from Enterococci

$\geq 16 \mu\text{g/mL}$

↓ common.

93

VISA (Vancomycin Intermediate (R) *Staph. aureus*)

↑ cell wall synthesis

4-8  $\mu\text{g/mL}$

VSSA (Vancomycin Susceptible S.A.)

$\leq 2 \mu\text{g/mL}$

MIC → Done for VRSA  
Neisseria  
Done by Broth Dilution

Q. Accessory Gene Regulator (agr)

MSCRAMM (Microbial surface component recognising  
Q adhesion matrix molecules)

↓

Protein A, Clumping Factor, Lectin.

Modified Hodge Test  
for carbapenemase (R)



# CONS

94

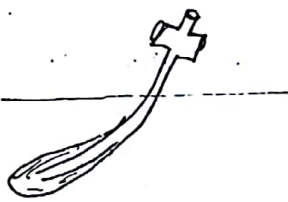
① *S. Epidermidis*  
75% infe<sup>n</sup>

Skin commensal → Lysostaphin

↓  
② *S. aureus*

R/F - IVC (central line)

Inter cellular adhesion → adhere to cellular tip



↓  
thin polysaccharide  
BIOFILM (300 nm)

↳ antiphagocytosis

Antibiotic (R)

C/F

Δ M/c cause of prosthetic valve endocarditis  
(early onset) - ~~H~~ < 12 months HAI

Late onset - *Strept. Viridians*

M/cc of CR BSI (catheter related Blood Stream Infection)

Δ of CRBSI → Culture of catheter tip (5cm)

Congo Red stain → biofilm.

Confocal microscope

↓

multiple focal point image

Superimposed

In situ Δ of CRBSI → BACTEC blood culture of 2

samples

- catheter

- peripheral vein



1/  $> 2hr$  difference in positivity  $\rightarrow$  CRBSI

95  
Differential to time test positivity

9/  $< 2hr$

$\downarrow$   
Bet<sup>n</sup> the +ve of 2 samples

Catheter Peripheral vein.

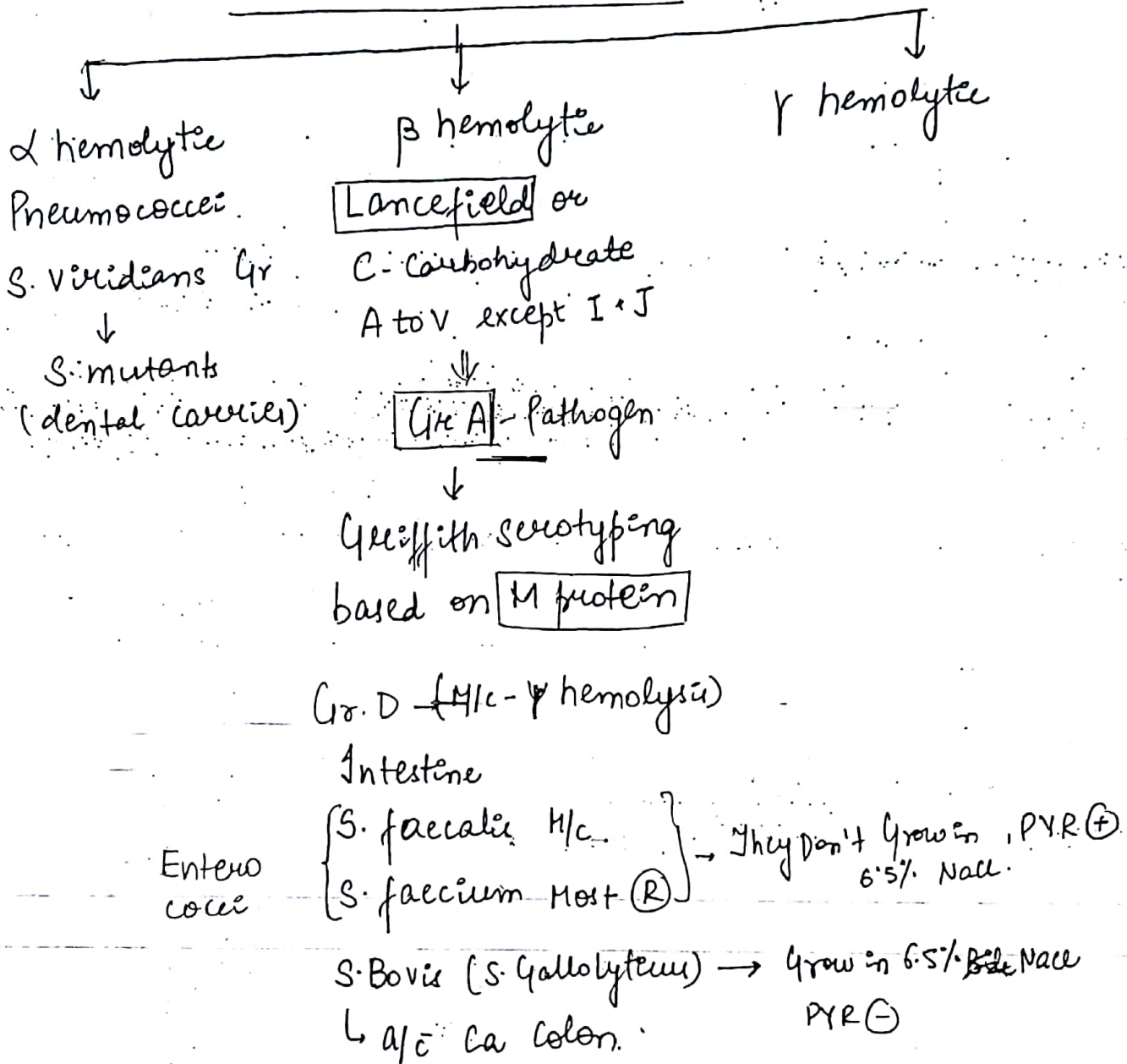
② *S. Saprophyticus*

UTI

Novobiocin (R)

# STREPTOCOCCUS

96



## GRP - A STREPTO

### Virulence Factors

1) Pyrogenic Exotoxin (erythrogenic)

↓  
Scarlet Fever → Rash (pastia's line)  
Sandpaper appearance  
Strawberry tongue  
M<sub>28</sub> - M/c serotype

## 2> M Protein

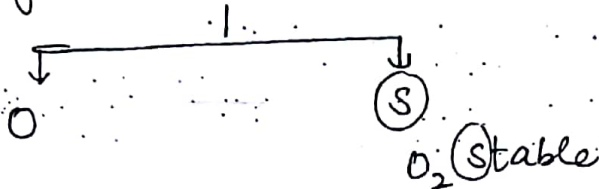
antiphagocytosis  
Serotyping



97

T & R protein  $\Rightarrow$  No virulence.

## 3> Hemolysin (Streptolysin)



$O_2$  labile  
active in reduced or  
anaerobic state

## ASO titre

ARF  $> 200$  IU

low in PSGN & Pyoderma

Tonsillitis strains  $M_1, 5, 12, 24$ .

Molecular mimicry  $\rightarrow$  Ab against cell wall protein of  
Group A strept. cross reacts w  
myocardium.

## 4> Streptodornase

destroys DNA

Anti streptodornase B  $\Rightarrow$  Marker for PSGN &  
Pyoderma

↓  
Skin pathogens

$M_{35, 43, 53-55, 59-61}$

5)

## Streptokinase

dissolve clot

used as thrombolytic agent → source is Gr C or *S. equisimilis*

98

6> Hyaluronic acid in capsule

7> Hyaluronidase → destroys tissue

Flesh eating bacteria

Gr A strepto M<sub>1</sub> to 5

↓  
Necrotising Fascitis

## Diagnosis

PYR (+)

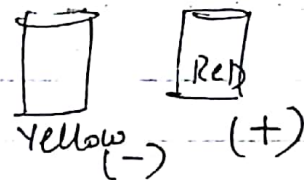
Bacitracin sensitive.

## PYR Test

Aminopeptidase enzyme

Releases free  $\beta$  naphthylamide

pyrrolidonyl  $\beta$  naphthylamide



PYR

(+) Gr A strept., enterococci

(-) Gr B strept., bovis



# GIR B STREPTO [S. GALACTRIAE]

99

↳ Bovine mastitis

20-40% female → genital tract



Neonatal Meningitis (direct spread)

H/c

Δ - Hippurate hydrolysis ⊕

CAMP ⊕ IOC

Hippurate Hydrolysis Test

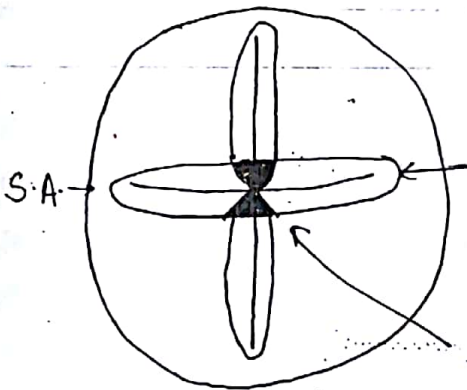
[Image].

Glycine + ninhydrine purple colour.

Hippurase enzyme acts on Hippurate

CAMP

Grp B strepto → stimulate Staph to release β toxin



Butterfly hemolysis

β hemolytic strepto

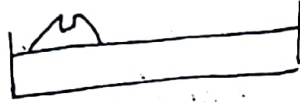
# PNEUMOCOCCUS

100

Lanceolate shaped diplococci  $\hat{=}$  capsule



Draughtsman colony (cannon coin)



## Virulence Factor

- 1) capsule
- 2) Ig A<sub>1</sub> protease
- 3) Pneumolysin } Not secreted
- 4)  $\alpha$  toxin

Autolysin  $\rightarrow$  destroys bacteria to release

C/F

M/cc of pyogenic meningitis  
" " community acquired pneumonia  
" " Hosp. acq. pneumonia (VAP)  
except

after 5-6 days } Pseudomonas  
MDR strain

" " Otitis media in  $<5y$

$\Delta$  - ① Inulin Fermentation (+)

② optochin ③  $\rightarrow$  screening test

③ Bile Solubility  $\rightarrow$  confirmatory

Rx

Meningitis → start ceftriaxone + vanco

101

↓  
Penicillin

other infect → start c penicillin

↓  
ceftriaxone + vanco

### Vaccine

Adult → PPSV (polyvalent polysaccharide vaccine  
23 seroprevalent strain)

↓  
High Risk

- splenectomy
- cochlear implant
- > 65 yrs
- chr. lung, kidney, liver, heart Ds
- immunocompromised.
- Diabetics
- Hospitalised pts c H/o smoking alcohol.

child - PCV13 [Pneumo conjugate vaccine]

2 - 24 months

OSLER TRIAD / AUSTRIAN SYNDROME :-

Pneumococcal endocarditis (Aortic valve) + Meningitis + Pneumonia

# ENTEROCOCCUS

102

↑ Resistant → UTI, HAI

↳ (R) to penicillin - hyperprod<sup>n</sup> of PBP 5

6.5% NaCl

9.6 pH

46°C

40% Bile

Δ → PYR (+)

Bile esculin hydrolysis (+)

S. Bovis → PYR (-)  
[Gallolyticus] = Bile esculin Hydrolysis (+)

VRE

plasmid

VAN A (nc) → (R) to both vancomycin + Teicoplanin

Van B, C, E → (R) to vanco only

Substitution of terminal

D alanine → D serine/  
Lactate

↓  
Elimination of Target



HLAR → high level aminoglycoside (R)

103

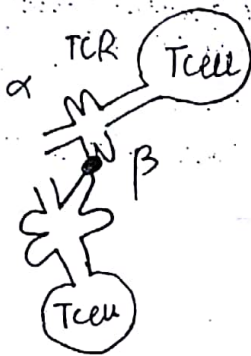
## SUPER ANTIGEN

MHC unrestricted

Bind to V $\beta$  region of TCR

↓  
polyclonal activation

↓  
20% of Total cells. [N] → 0.1% of total cells  
↓  
↑↑ Cytokines. [in monoclonal activation]



## Pathogen showing superantigen

- 1) Staph aureus
- 2) GHA strepto
- 3) M.tb. including BCG
- 4) Rabies
- 5) HIV
- 6) EBV

IgA → Mucosal Affinity

≤ Ig doesn't cross placenta

a) Ig G<sub>1</sub>

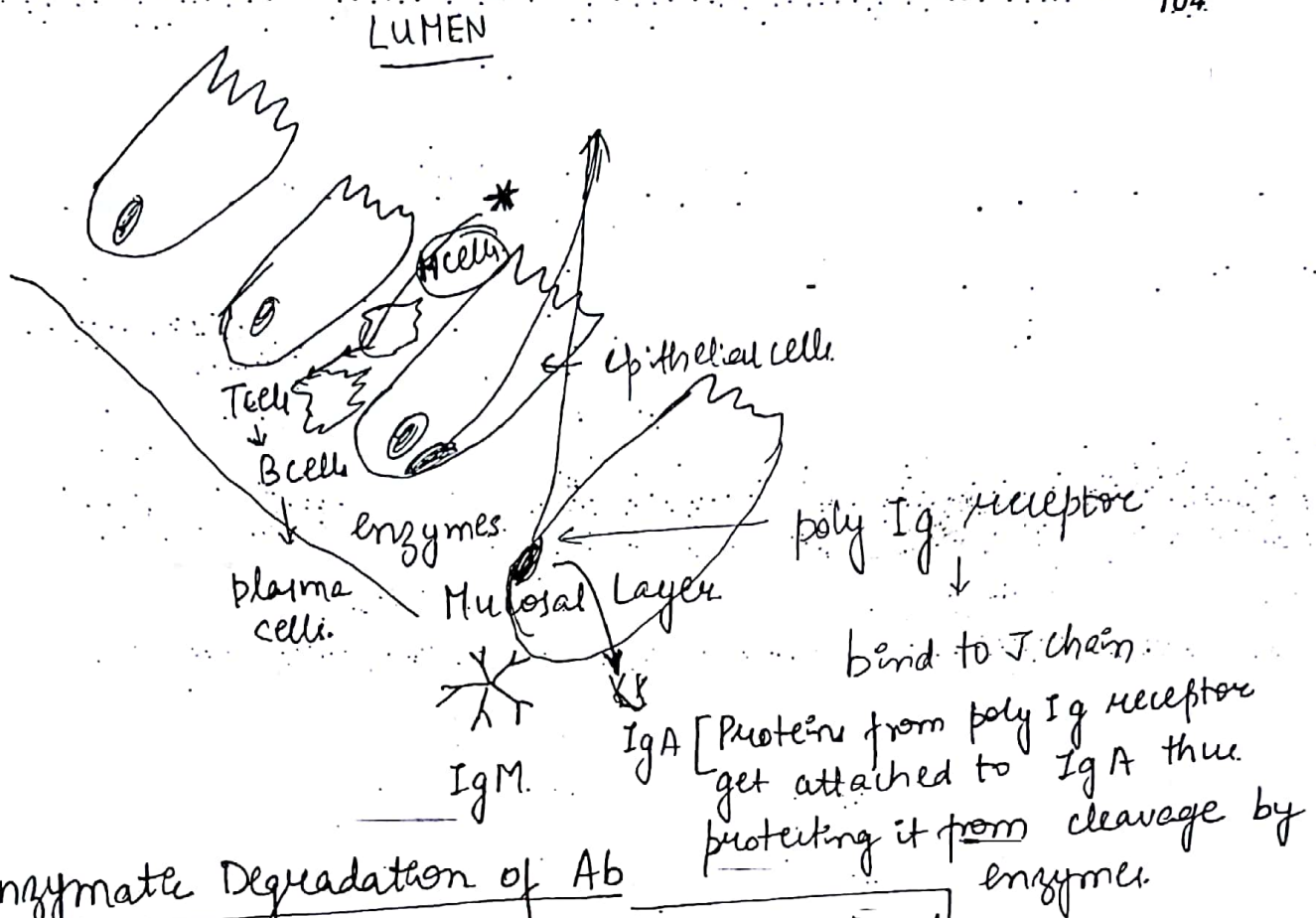
b) Ig G<sub>2</sub>

c) Ig G<sub>3</sub>

d) Ig G<sub>4</sub>

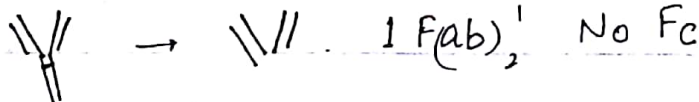
→ placenta doesn't have receptor for  
F<sub>c</sub> portion of Ig G<sub>2</sub>

[Receptor mediated Trans cytosis of Ab]

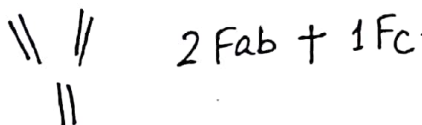


### Enzymatic Degradation of Ab

Pepsin → cleaves below di-sulphide Bond.

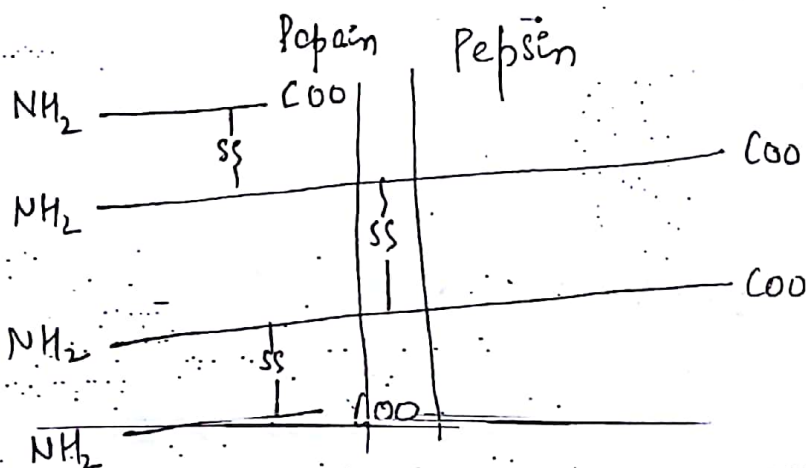


Papain → cleaves above disulphide Bond



secretory portion

↓  
It derived from part of poly Ig receptor.]



H chain is cleaved → Isotype func<sup>n</sup> is lost

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L chain is intact → Idiotype intact

Ag. Binding occurs.

Valency (monomer)

Pepsin → unchanged.

Papain → 2 → 1.

2ME - 2 Mercaptoethanol → cleaves disulphide bond

2H + 2L chains

Idiotype, Isotype all lost

DIFFERENTIATE S. AUREUS FROM MICROCOCOCCUS

Micrococcus

Obligate aerobe

Non-pathogen

Tetrad.

Skin commensal

Hugh & Leitson's oxidation Fermentation Test

Fermentation

↓  
S. aureus

Oxidation.

↓  
Micrococcus

Diff in Blood Agar

Group A strept → Bacitracin (S)

Pneumococci → optochin (S)

24/2/18

\* Non-Streptococcal Catalase -ve Gram +ve Cocci 106

→ Pediococcus + Leuconostoc → Vancomycin Resistant

→ Abiotrophia + Granulicatella species

nutritionally variant streptococci → require Vit B6 ⊕

Doc → Gentamicin + Penicillin (to avoid resistance)

\* S. Anginosus Gr

Agglutinate A, C, G, (F) anturea

VP test ⊕

Buttercrotch, or caramel odour.

## GRAM +ve BACILLI

### ① LISTERIA MONOCYTOGENES

1> Only Gram +ve Bacillus ⊆ has Endotoxin.

2> Intracellular pathogen.

Int. A & B toxin → helps in internalisation.

3> (LLO) Listeriolysin O secreted by Listeria destroys phagosome & thus escapes phagocytosis.

Listeriolysin + Cytotoxin ⇒ helps in escaping phagocytosis

→ Haemolysin  
show phemolysis



### 3) Actin Filaments

help in intracellular & intercellular motility

↓  
BLEB Formation

[Image]

4) Cold Growth. ( $2-8^{\circ}\text{C}$ )

5)

C/F → Neonatal Meningitis

↓  
Early Onset

Granulomatous Infantile

Intracellular Transmission.

Mother is asymptomatic

Disseminated Cond<sup>n</sup>

↓  
Late Onset

10-30 days after birth

Neonatal Meningitis

Mother is asymptomatic

Spread through faecal  
contamination by health  
care workers.

Adult ⇒ Food Poisoning

By consumption of Refrigerated food.

6) Δ → ① Blood Agar → β hemolysis

② CAMP test → (+)

③ Anton test → (+)

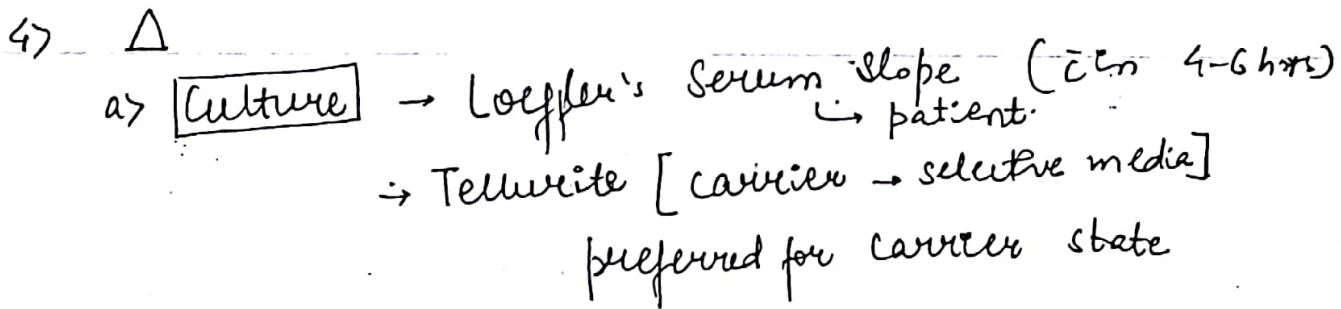
④ Tumbling Motility at  $21^{\circ}\text{C}$ .

⑤ Ab detection

⑥ PCR (↑ sensitivity) — Good method nowadays.

**108**

- It depends on Iron Conc<sup>n</sup>.  
[0.1 mg/lc]



If not specified patient or carrier  $\rightarrow$  go for LSS

→ Hogle, Tinsdale  
Miss Susan Water

b7 Specimen → Throat Swab.

GRAVIS

Daisy Head Colony

More virulent

INTERMEDIUS

Frog Egg Colony

~~POACHED EGG COLONY~~  
MITIS

Poached Egg Colony

c) Microscopic Exam of Throat Swab  
↳ for toxigenicity.

i) Albert Stain

ii) Neisser Stain

iii) Ponder

Metachromatic

or

Volutin

or

Baker Ernest granules.

↓

Pathogenic.

d) Toxigenicity

In vivo → Guinea Pig  
(250-350gm)

Subcutaneous

Intracutaneous

s/c inj<sup>n</sup> of test strain

↓  
death in 48 hrs

Use Control → s/c inj<sup>n</sup> of  
test strain + 500 IU of  
diphtheria antitoxin intraperitoneally

Protective  
Dose

4 Guinea Pig ⇒ 3 for test + 1 control  
used

Rabbit - 500gm

Guinea Pig - 250gm

Mice - 20gm

I/c inj<sup>n</sup> of test strain  
↓ after 4hrs

50 IU of diphtheria  
antitoxin intraperitoneally

↓

Cutaneous Lesions in  
48-72 hrs.

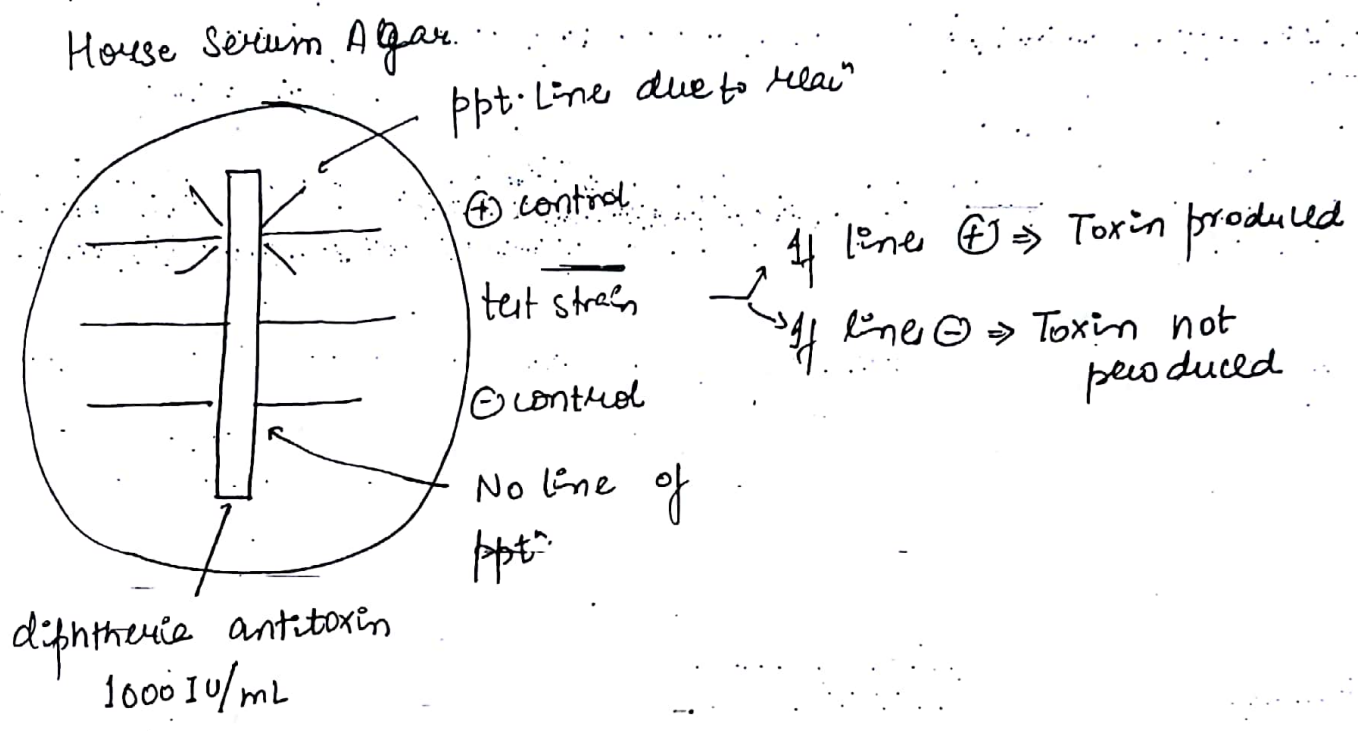
Advantage - 10 test  
performed on 1 guinea  
pig

1 IU of Antitoxin = Min. amount Required  
to neutralise 100 Fg of toxin.



In Vitro → Elek's Gel precipitation

Double Diffusion in 2 directions  
[Ouchterlony Procedure]



- *Corynebacterium Jeikeium* ⇒ MDR
- " *Urealyticum* ⇒ urease +ve (UTI)
- *Acetobacterium haemolyticum* ⇒ Reverse CAMP test (+)

SCHICK TEST (Neutralization Test)  
for susceptibility

Toxin

(one arm)

Heat inactivated toxin

(other arm)

- Positive (susceptible) (+)
- Negative (Immune) (-)
- Pseudoreac<sup>n</sup> [immune but Hypersensitive] (-)

- (-)
- (-)
- (+)



Combined Reac<sup>n</sup>  
(Susceptible + Hypersensitive)

(+)

(+)

111

Toxoid is safe than heat inactivated Toxin.  
So, no fear of anaphylaxis

DIPHTHERIA TOXOID

5-25 Lf units/dose [0.1ml]

[Lf = Limes  
flocculation]

TT → 10 Lf

dT → low dose → 1-2 Lf  
↓  
used in adults.

## BACILLUS ANTHRACIS

Zoonotic Disease

Non-Motile.

Seasonal outbreak - summer season.

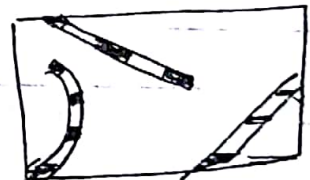
Dry Grass ← feeding Cattle

↓  
gets injured

(spores + nt in soil) Spores enter

↓  
Haemorrhage

↓  
Death.



Cutaneous Anthrax  
 Hide porter Disease  
 ↓  
 Malignant pustule  
 ↓  
 Black Eschar

INCIDENCE

MORTALITY

45%

20%

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Pulmonary Anthrax  
 Wool sorter

5%

45%

Intestinal Anthrax

rare

45%

### Virulence Factor

1) Capsule

2) Toxin

Plasmid

3)

→ Oedema factor (↑ cAMP)

→ Protective factor } cytotoxic

→ Lethal factor


### Diagnosis

Transportation of sample → Triple layer packaging  
 (used in infectious sample)

Culture on PLET Media

[Polymyxin, Lysozyme, EDTA, Thallus acetate]

↓

Medusa Head Colony → 

String of Pearl → oooooo

Bamboo stick or Box Car Appearance

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B. anthracis  
(obligate aerobe)

also seen in Clostridium Perfringens.  
(obligate anaerobe)

Inverted Fir Tree → on Gelatin stab culture



McFadyen Reac<sup>n</sup> → for capsule

Confirmation

1) γ phase lysis  
or

2) MLST (multilocus sequence typing)  
by PCR

[to distinguish other spore bearing, aerobe organism]

Bioterrorism → Category 'A' agent

B. anthracis	→ Most potent
Yersinia Bacter	
C. Botulinum	
Francisella tularensis	
Small Pox	
Agent of Viral Haemorrhagic fever	→ epidemiologically important.

B. CEREUS

Motile

- causes food poisoning - By RICE

## Food poisoning

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### Emetic

vomiting

preformed toxin

< 6 hrs IP

↑↑ bacilli in stool

### Enteric

diarrhoea

> 12 hrs IP

↓↓ bacilli in stool

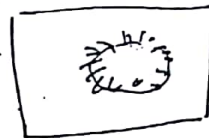
Δ - Culture - MYPA media

(mannitol Yeast Phenol Red novobiocin agar)

## ACTINOMYCES

Gram +ve Bacilli [Image]

Filamentous



Obligate Anaerobe

A → Actinomyces

B → Bacteroides [Gram -ve]

C → Clostridium

No ~~by~~ superoxide Dismutase

Δ No Peroxidase

actinomyces

Gram +ve Branching filamentous rods

Nocardia



# Actinomyces

Anaerobe

Non Acid Fast

Endogenous Infect

↳ oral cavity

Lumpy Jaw - M/C

GIT

Genitourinary Tract

Thoracic Actinomyces

↳ aspiration of oral secretions

Δ of Actinomyces

Actinomyces involvement of pelvis occurs most commonly in association with an IUD.

↳ Sulphur Granules

filamentous bacteria in peripheral clots.

Ag - Ab complex.

↓  
Sunray appearance.

MYCETOMA

swelling

sinus

granules

Actinomycetoma → actinomyces, Nocardia + streptococcus

Eumycetoma → Fungi pigmented

Botryomycosis → S. aureus (M/C)

Actinomycetoma

acute inflammatory cond

Purulent D/C

white or yellow sulphur granules

except actinomadura pelletiere

↳ Red granules

# Nocardia

115

Aerobe

Acid Fast (1% H<sub>2</sub>SO<sub>4</sub>)

Exogenous Infect

Nocardia	Mycobacteria
entangled red bacilli	Long, slender Beaded

Eumycetoma

Chronic

Purulent D/C

Serous D/C

Brown & Blue stain.

Gram stain.

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27 CULTURE → Molar Tooth Colony  
Spiderly colonies

### Δ of NOCARDIA

1° infection in Lungs → Brain & Kidney Abscess.  
subcutaneous Infection → Mycetoma

- Gram stain

- Acid Fastness

- Culture using Paraffin Bait Technique

BACTEROIDES FRAGILE [Gram - ve  
Anaerobe]

### 1> Virulence Factors

Capsular polysaccharide

Protease & neuraminidase

- Cytolysin

Enterotoxin

LPS - 1000 times less Biologically potent

Drug resistance to  $\beta$  lactamase

## 2) LEMIERRE'S SYNDROME

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Jugular thrombophlebitis

*Fusobacterium necrophorum* → Bacteroid species

Lung emboli sepsis

3) Δ → culture Media

- a) Trypticase Soy Agar
  - b) Schaedler's blood agar
  - c) BHI (Brain Heart Infusion)
- Kanamycin

## CLOSTRIDIUM

Obligate anaerobe

No  $O_2$  / ↓ Eh (Redox Potential)

### C. TETANI

Drumstick appearance

Virulence Factor -

Tetanospasmin

No virulence

Tetanospasmin

- plays role in virulence
- ⊖ GABA + glycine
- ↓
- ⊖ of presynaptic receptors
- ↓
- Spasmodic  
Spastic paralysis

Δ - Robertson Cooked Meat Broth

Thioglycollate Broth



Blood Agar



Macintosh Field.

→ [Image]

(Molybdenum catalyst)

Candle Jar  
↓  
for capnophiles

BI for checking  $O_2$  free environment  
in Macintosh Field ⇒ 'Pseudomonas'



It does not grow if completely  $O_2$  free

GAS PAK JAR

→ Better, safe

Prevention : Active Immunisation + Passive immunisation.

PEP for tetanus

Immunity Category

Simple wound

Contaminated wound

Cat A

Nothing Req'd

Nothing Req'd

Cat B

Toxoid 1 dose

Toxoid 1 dose

Cat C

Toxoid 1 dose

Toxoid 1 dose + HTIG

Cat D

Toxoid complete dose

Toxoid complete dose  
+ HTIG.



A - taken complete course of TT/Booster in part 5 yrs  
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B - " " " " " in part 5-10 yrs

C - " " " " " in part >10 yrs

D - Not taken complete course of TT/booster in part 5 yrs.

Simple wound - <6 hrs, clean, non-penetrating, negligible tissue damage.

Booster recommended every 10 years.

1<sup>o</sup> Immunisation - 1st 3 doses on 6, 10, 14 weeks.  
Safe vaccine.

Pertussis vaccine - causes fever as cellular component is used.

## C. PERFRINGES / WELCH

12 toxins

$\alpha$  toxin  $\rightarrow$  Most virulent

$\downarrow$   
lecithinase c / phospholipase

C/F

① Gas Gangrene

② food poisoning

$\rightarrow$  C. perfringens 60%

C. novyi / septicum 40%

Type A

Type C

Drambrand.

Germany

Pigbel

Papu New Guinea

Enteritis Neurotoxic

Endemic

World wide

Neurotoxic enterocolitis

$\alpha$  toxin  $\rightarrow$  ileum  $\rightarrow$  Necrosis + Gas

$\alpha$  toxin  $\rightarrow$  jejunum  $\rightarrow$  Necrosis + Gas

Neurotoxic enterococci

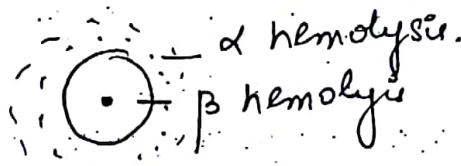
120

↓  
Fice. In The Belly.

1 - ① Target Sign.

around clostridium  $\Rightarrow$  zone of  $\beta$  hemolysis due to  $\theta$  toxin.

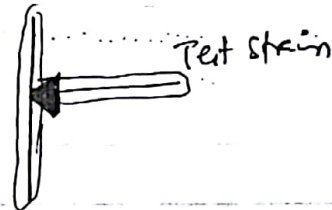
zone of  $\alpha$  hemolysis due to  $\alpha$  toxin



② Reverse CAMP Test (+ve)

- Group B streptococcus used for
- \* Test strain is inoculated.

Arrow type hemolysis.



Bow Type hemolysis

Toxin of clostridium goes to strepto + causes more hemolysis.

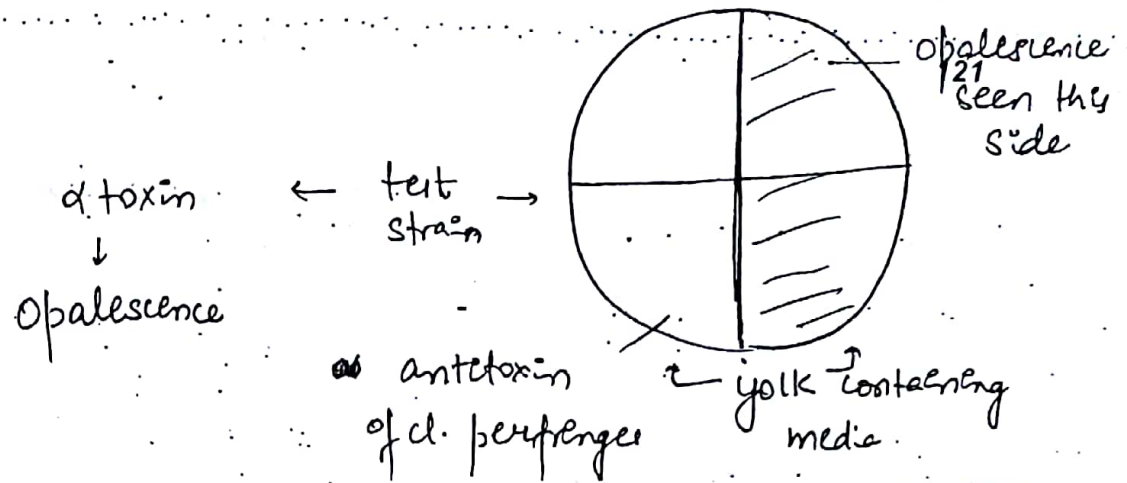
Group B Streptococcus

Toxin of strept. goes towards clostridium & causes more hemolysis

③ stormy fermentation on litmus milk

④ Nagler Test

Volk containing media



## C. BOTULINUM

### \* Virulence Factor

Toxins → all are neurotoxins. except  $C_2$  (enterotoxin)

Botox Injec<sup>n</sup> → anti-aging

Bioremediation

### \* Pathogenicity -

1) ⊖ Ach → Flaccid Paralysis.

2) food poisoning

preformed toxin. in home made canned food.

IP - 8 to 36 hrs G.

3) Floppy child syndrome  
spores (HONEY)

Infant gut microbiota starts developing in 4 hrs.

4) ~~Wound Botulism~~

due to spores.

# $\Delta$ - Toxin Detection

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## Cl. DIFFICILE

Cause pseudomembranous Colitis due to Clindamycin.  
cephalosporin [2<sup>nd</sup> < 3<sup>rd</sup> generation] (MC)

### \* Virulence Factor

Toxin  $\left\{ \begin{array}{l} \text{A} - \text{enterotoxin} \\ \text{B} - \text{cytotoxin} \end{array} \right\}$  Both toxin glycosylate GTP  
binding proteins of Rho subfamily  
 $\downarrow$  protein synthesis

### \* Pathogenicity -

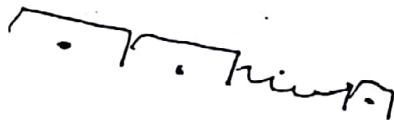
Sammit Lesions



Pseudomembrane



Neurosis



NO Gross Blood in stool.

\*  $\Delta$



Test	Sensitivity	Specificity
Stool culture Best test for confirmation. (Gold Std)	++++	+++ <sup>123</sup>
Cell culture cytotoxin test on stool	+++	(++++)
ELISA for toxin A + B in stool	++	- +++.
ELISA for C difficile common Ag in stool	++++	+++
Best Test NAAT for toxin A + B gene in stool	(++++)	(++++)
Colonoscopy or sigmoidoscopy	+	++++

1<sup>st</sup> Rx (1) DOC - Metronidazole  
or

Vancomycin → ↑ effective → used in severe cases.  
To prevent incidence of VRE.

2<sup>nd</sup> Fecal Transplantation

# GRAM -ve Cocci

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## NEISSERIA

Intracellular, diplococci

Oxidase (+)

Media req. for growth - chocolate agar  
Thayer Martin  
New York City  
Martin Lewis

Candle Jar useful → as they are capnophilic

Oxidase Test

Kovac Reagent [p-phenylenediamine dihydrochloride]

↓ cytochrome oxidase

Blue (indophenol)

(+) → Pseudomonas

Aeromonas

Neisseria

Campylobacter

Haemophilus

\* (-) → Enterobacteriaceae

# N. MENINGITIDIS

125

Virulence factor

- 1) Capsule
- 2) Endotoxin
- 3) ~~Pili~~ Pili

C/F  $\Rightarrow$  Pyogenic Meningitis + Rash.  
leads to outbreak.

Waterhouse Friedrichson Syndrome [due to endotoxin]

Serotypes A, B, C  $\Rightarrow$  outbreak.

Y, W<sub>135</sub>  $\Rightarrow$  Sporadic cases.

X, Y, Z9E  $\Rightarrow$  in HIV pts.

others  $\rightarrow$  carriers

$\downarrow$   
Nasopharyngeal 5-10%.

$\downarrow$   
during outbreak 80-90%.

chemoprophylaxis - Inj<sup>n</sup> ceftriaxone > cipro > Rifampicin.

Affects European countries. In India - Meghalaya.  
 $\downarrow$   
we use cipro.

Vaccine - polyvalent polysaccharide vaccine

$\downarrow$   
A, C, Y, W<sub>135</sub>.

(Gr B)  $\rightarrow$  Inj<sup>n</sup> + out deficiency of Late complement  
proteins properdin.

$\rightarrow$  M/c Inj<sup>n</sup> in infants

$\rightarrow$  capsule is non-immunogenic

Group B vaccine: based on subcapsular antigens.

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not in cell wall.

outer membrane vesicles.

Neisserial adhesion factor A.

Factor H binding protein.

neisserial heparin binding antigen

\*Rapid Carbohydrate Utilisation Test :-

ONPG test [O-Nitrophenyl  $\beta$ -D-galactopyranoside].

$\beta$  galactosidase

galactose + O-Nitrophenyl.  
[yellow].

N. lactamica  $\rightarrow$  ferments lactose unlike other N. species

N. Meningitis  $\Rightarrow$  Maltose fermentation.

N. Flavescence  $\Rightarrow$  F/Sucrose "

N. Gonorrhoea  $\Rightarrow$  glucose "

## MORAXELLA CATARRHALIS

Cause - Otitis Media

Sinusitis.

COPD - AE

$\Delta$  - - Hockey Puck Sign

colonies can be slid across the agar surface.

no disruption

- Lacks Carbohydrate Fermentation
- Produce DNase
- Butyrate esterase used to differentiate from saprophyte
- Neisseria



# Drug Resistance to $\beta$ Lactamase.

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## N. GONORRHOEA

### Virulence Factors

- 1) Pili Adhesion.
- 2) Opa (Protein II) - adhesion.
  - transient ↓ in CD4
- 3) Porin (protein I)
  - endocytosis, invasion
  - ⊖ Complements
- 4) Rmp (protein III) - Blocks Ab block. effect of bactericidal.
  - Ab to porin + LOS leading to reinfection
  - (lipooligosaccharide)
- 5) Ig A1 protease
- Fbp (ferric Binding protein).
- Lip (HB)

- 6) LOS - endotoxigenicity → Chronic Infection.
- Re Pili, Opa, LOS express Phenotypic variation
- Por of different strains express different Ag typing
- Proteins inside human body doesn't change → they remain same.
- ↳ It shows variation in different strains.

### Resistance

PPNG -  $\beta$  lactamase  
(M/c) (plasmid)  
Common

producing strain of N. gonorrhoeae  
encoded

CMRNG - chromosomal encoded high level resistance to penicillin.

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have

TRNG - High Level Tetracycline resistance ( $MIC \geq 16 \text{ mg/L}$ )  
- tet M gene on conjugative plasmid.

Ceftriaxone resistant - chromosomally encoded pen A gene  
expressing PBP 2a.

Gonococcus doesn't affect vagina.

I.P. - 3-4 days. [Chlamydia - 7 days]  
MIC

Chlamydia diagnosed  
by NAAT

GRAM -ve BACILLI

ENTEROBACTERIACE

① Ferment Glucose

② Motile / Non-Motile

↓  
peritrichous flagella

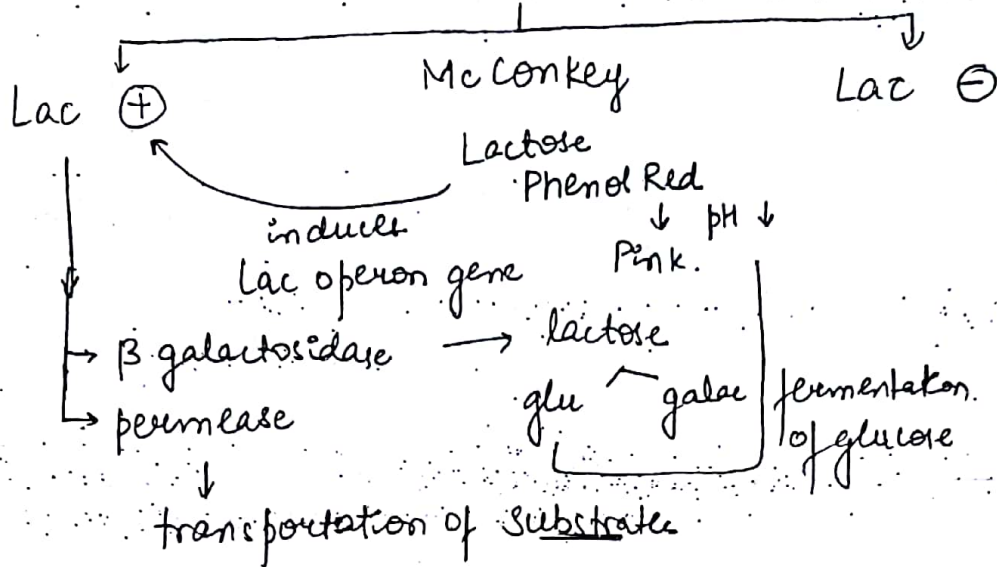
③ Catalase (+) except *S. dysenteriae* Type I

④ Oxidase (-)

⑤ Reduce nitrate to Nitrite

# Enterobacteriaceae

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E. coli - [except Enteroinvasive E. coli]

Klebsiella

Enterobacter

Serratia

Edwardsiella

Citrobacter

Arizona

Providencia

Campylobacter

Shigella sonnei

Salmonella

Shigella

Proteus

Yersinia

Enteroinvasive E. coli

Triple Sugar Iron Agar Media (TSI)

[glucose, lactose, sucrose]  
disaccharide

1 : 10 : 10

aerobic

Slant

butt.

anaerobic

Slant

→ oxidative deamination

peptides (AA)

broken down to  $\text{NH}_3$

↑ pH. [Red]

Butt → fermentation → ↓ pH [yellow].

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Lactose (+)



Initially, glucose fermentation occurs

↓  
yellow colour.



Later glucose is finished.



Then Lac (+) organism causes

Lactose metabolism



So pH ↓



Again Yellow

Lac (-)



Initially glucose fermentation occurs



yellow colour



Later glucose finishes



Then Lac (-) can't metabolise Lactose



No new acid production

So, oxidative deamination continues. → out any neutralisation.



↑ pH



Red colour

Lac (+) = Acid  
Acid

Lac (-) = Alkaline =  $\frac{K}{A}$   
Acid



Non-Fermenter

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Non-fermenter =  $\frac{\text{Alkaline}}{\text{Alkaline}}$

Oxidative Deamination occurs.

Vibrio - Lac<sup>-</sup>  $\left. \begin{array}{l} \text{glucose} \\ \text{Sucrose} \end{array} \right\}$  fermentation (+)



for Vibrio - TSI + McConkey Req

as in TSI resemble Lac<sup>+</sup> organisms

H<sub>2</sub>S producing Enterobacteriaceae

TSI media since contain Fe

$\text{Fe} + \text{H}_2\text{S} \Rightarrow \text{Black colour} \Rightarrow \text{BLACK COLONY}$

Salmonella

Proteus

Citrobacter

Edwardsiella

Media used

- 1) Bismuth sulfite citrate sulfide
- 2) Deoxycholate citrate agar (DCA)
- 3) Xylose Lysine ~~deoxy~~ deoxycholate (XLD)
- 4) Salmonella shigella Agar (SS)
- 5) Hekton enteric agar (

- 6) <sup>Fe</sup> Lysine agar
- 7) TSI, <sup>Fe</sup> Kligler Fe Iron (KIA)

- 8) SIM (Sulfide Indole motility medium)
- 9) Lead Acetate Agar

tube

KIA Contains - glucose: Lactose  
1:10

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It is used in vibrio non-endemic area as it  
doesn't contain sucrose as sucrose is for vibrio

## E. COLI

UTI - ~~fib~~ fimbriae P, Fe, S, Type 1.  
Lower serotypes O<sub>1</sub> O<sub>2</sub> O<sub>4</sub> O<sub>6</sub> O<sub>7</sub>

→ Screening -

M/E

Guiness Nitrate Test

Conf<sup>n</sup> { culture of urine.  
Significant ~~bacteremia~~ with  
bacteremia  
Midstream urine  
Suprapubic aspiration.  
KASS CONCEPT

$> 10^5$  c/fu/mL

except → Urine +ve org. }  $10^2$  to  $10^5$   
Catheterised patient

Urine culture

std. loop technique

Miles & Mitty

Quantitative

Semi-Quantitative

1 mL of urine in media

4mm internal diameter

0.001 mL approx.

Counter Counter

Colony counter



Media -

1) McConkey + Blood Agar

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↳ used so that *Staph aureus* if present

2) CLED (cysteine Lactose electrolyte Deficient media)

- differential media
- prevents swarming of proteus
- ~~promotes~~ promotes growth of *Staph* + *Candida*  
Q [enriched media not required]

## Diarrhoea

Serotype

Virulence Factor

1) STEC  
EHEC

— Lambda like Stx1 or Stx2 encoding  
bacteriophage

2) ETEC

CFA, LT + ST (plasmid)

3) EPEC

EPEC adherence factor (plasmid)  
Locus for enterocyte effacement (LEE) -  
chromosomal pathogenicity island

4) EIEC

Invasion, Intracellular spread.  
cell to cell transmission (plasmid)

5) EAEC

Adherence + toxin genes (chromosomal  
plasmid)

**EPEC**

- Paediatric  
H/C infant children.

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Non-inflammatory Diarrhoea  
& Adhesion on HELA/HEP cell lines

↓  
Enteroadhesive E. coli

~~EHEC~~

Δ - Serotyping

**ETEC**

- T- Traveller's Diarrhoea H/C

CFA

Toxins

LT (labile)

↓  
↑ cAMP

Δ - Rabbit ileal loop  
ligation test



Ballooning

**BIKEN test**

(ppt)

ST (stable)

↓  
↑ cGMP

Infant or mice ileal  
loop ligation

Aggdetec by ELISA  
using mouse Ig

\* Serotyping for ETEC.

ETEC causes non-inflammatory diarrhoea



EIEC → atypical E. coli  
Non-motile / Lac<sup>-</sup>

135

Inflammatory Diarrhoea

Δ - Sereny Test [conjunctivitis in Rabbit eye]  
serotyping

EHEC → Inflammatory Diarrhoea & Blood.  
due to stx (shiga like toxin) or verocytotoxin

↓  
haemorrhagic colitis

HUS → EHEC (H/C)  
90% association.

Stx<sub>2</sub> > Stx<sub>1</sub>.

M/C cause of outbreak.

Δ - For Screening ⇒ Sorbitol MacConkey media

↓  
EHEC doesn't ferment sorbitol

serotyping

EPEC → BRICK STACK PATTERN  
Non-inflammatory Persistent Diarrhoea

# KLEBSIELLA

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Necrotising Pneumonia & Empyema

Lower Lobe → Lobar pneumonia

HAI. (hosp. acquired)

Hypervirulent *Klebsiella pneumoniae* (hvKP)

1) Hypermucoviscous phenotype

2) ↑ dissemination

3) String Test (>5mm long) → also seen in *Vibrio*

4) *Klebsiella* - lysine decarboxylase

— *E. coli* —

Motility (+)

Urease (-)

Mucoid colony (-)

IMViC ++--

Indole

Indole Test

~~Indole~~ Tryptophan

↓ Tryptophase

Indole; Pyruvic acid -  $\text{NH}_3$

↓

red complex aldehyde group.

in KOVAc or Ehrlich reagent

*Klebsiella* —

(-)

(+)

(+)

-- ++

- (+) → E. Coli
- (-) → Klebsiella  
Enterobacter.  
Hafnia  
Serratia.

**Methyl Red**

pH indicator - Red at 4.4.

- (+) for E. Coli

**Voges Proskauer**

Acetoin detected.

- (+) for Klebsiella  
Enterobacter  
Hafnia  
Serratia.

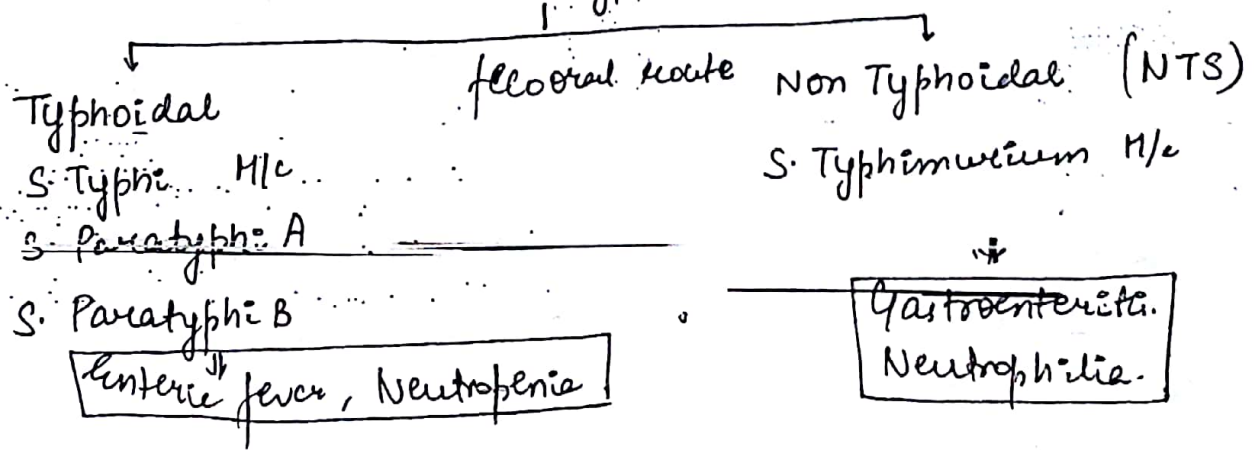
**Citrate**

utilisation of citrate  
When citrate utilised, Green → Blue colour change

SALMONELLA

Salmonella enterica subspecies enterica

Serotypes



Serotype - motile except *S. Gallinarum* +  
*S. Pullorum*.

138

## NTS

Salmonella → Toxin → causes endothelial intestinal  
epithelial damage.

↓

IL8

↓

Neutrophilia

— No blood or mucus

Pus (+) in stool.

R<sub>x</sub> - not required  
no antibiotics.

Since antibiotics will lead to ↑ fecal shedding  
↓  
'outbreak'.

## ENTERIC FEVER

Salmonella multiplies in Peyer's patches [MALT]

↓

Spleen, Lymph node,

Bone marrow.

↳ it gets blocked  
leading to neutropenia

Neutropenia

• Hepatosplenomegaly

Lymphadenopathy



Other features  
~~Confirmation~~

Rose spots

Bradycardia

Step ladder fever.

Pea Soup stool [constipation/diarrhoea]



For confirmation

Blood culture - 1st + 2nd week 1:10 dilution. 139

Widal test - 3rd wk onwards.

### WIDAL TEST

Tube agglutination.

To detect O & H Ab in pt serum using Widal Ag.

T<sub>O</sub> - 'O' Ag of S. Typhi

T<sub>H</sub> - 'H' Ag of S. Typhi

A<sub>H</sub> - 'H' Ag of S. paratyphi A

B<sub>H</sub> - 'H' Ag of S. paratyphi B.

'O' Ab appears early 'H' Ab persists longer.

'O' agglutinin is granular → round bottle FELIX TUBE

'H' " " fluffily → concave DREYER TUBE

Mirror is used for looking at the agglutination at the bottom.

16 tubes are used (4x4)

Widal Ag extraction - (O-901 strain of salmonella used)

'O' Ag → Heat stable

Smooth strain (LPS = OAg)

grow in phenol Agar. H-O variation. [loss of flagella]

ethanol / chloroform

Not total loss

'H' Ag. → Heat labile

Rough strain (No LPS - No Ag)

140

S → R variation (Loss of virulence)

"old culture"

autoagglutinable.

grow in Craigie Tube.

'H' antigen and microste.

salmonella



semisolid  
agar media

→ formalin is used for killing

Factors affecting Widal

1) Endemicity

(single serum sample)

cut off titre

'O' Ab  $\geq$  1:320

'H' Ab  $\geq$  1:640

④

4 fold rise in titre

2) Anamnestic Reac<sup>n</sup>

Transient Rise in widal Titre due to some other  
infection in apparently a previously salmonella  
infected pt.

IgG is responsible for it

3) False +ve Reac<sup>n</sup>

due to some other infect<sup>n</sup>.

4) Rx

5) Vaccination.

— TAB (killed) , Ty 21a (live)] polyvalent

Vi - polysaccharide - monovalent

↓  
Does not affect widal titres.  
from 2yrs to any age group.

141

Rx Ciprofloxacin - DOC  
2nd line - ceftriaxone

### CARRIER

By Bile culture

Stool culture

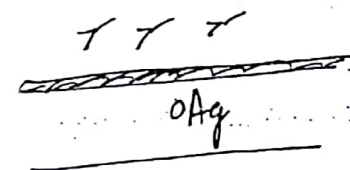
Vi Ab detection using viAg (source is Citrobacter)

Serotyping in salmonella  
Kauffman White Scheme.

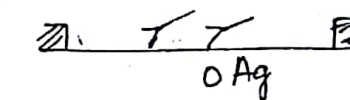
False negativity while  
serotyping is removed by  
V → W variation achieved  
by heating

Best specimen - Bone Marrow culture Non-① agglutinable

(painful but ↑  
sensitivity)

(a) 

 V forms fresh isolate.

(b) 

W forms

① agglutination.

# SHIGELLA

142

- Non-Motile
- ~~Produce Gas~~ (due to ~~pyruvic~~ formic acid)
- No Gas:- can't cleave formic acid.  
(anaerogenic)
- Infective Dose - 10-100 bacilli
- Cause outbreak

## S. DYSENTERIAE

chromosomally encoded toxins

↓  
verocytotoxin (ctx)  
enterotoxin

neurotoxin (acts on blood vessels)

12 serotypes

3.

## S. FLEXNERI

6 serotypes - X & Y Variant

More common in India

## S. BOYDI

18 serotypes

## S. SONNEI

no serotypes

→ O antigenically homogeneous.

MOST common worldwide

✓ Colicin Typing (Bacteriocin Typing)



Invasion → By Virulence marker Ag (plasmid)

143

Δ - Stool Culture. (Salmonella + shigella)

\* Enrichment media [liquid + inhibition].

↳ Yersinia thionate Broth

Selenite F Broth.

SS Broth

\* Selective media → media for  $H_2S$  detection

### PROTEUS

Swarming  
Pleomorphic

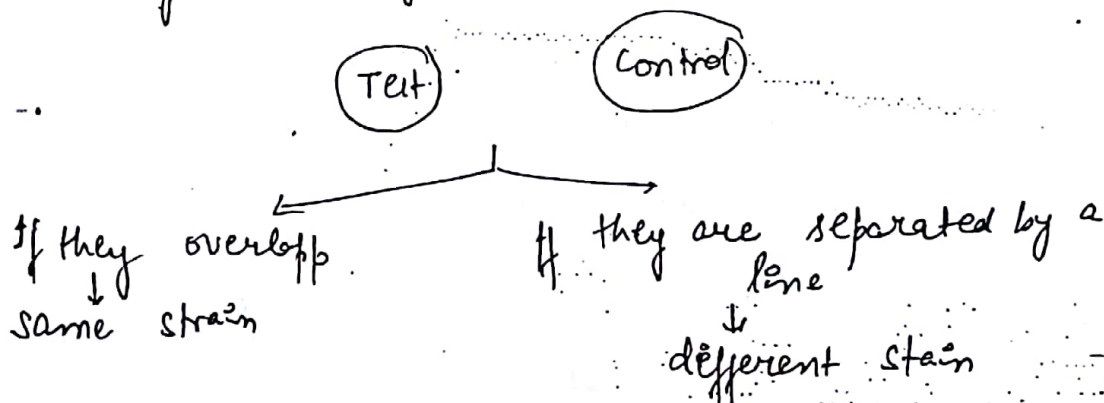
produce urease → causes UTI + calculi  
(Struvite)

Δ - PPA test (Confirmation for Proteus, Providencia + Morganella)

↓  
Phenyl pyruvic acid

Diene phenomenon for typing

↓  
using swarming



# YERSINIA PESTIS

144

VF:- Toxin → No virulence Q

VW. antigen

F<sub>1</sub> protein (plasmid)

Coagulase

Phospholipase

Virulence +

helps bacilli to survive at  
27°C in flea midgut

## Plague (Black Death)

↓

- Bubo

- pneumonic bubo → if enters lung

- Septicaemic

- outbreak

## Endemic Area

① Beed - Latour

② Kohlar

③ Rohru (shimla)

④ Uttarakhand.

A - Wayson Stain

safety pin Bacilli

due to bipolar staining

Ghee Buth - Stallactite growth.  
at 27°C

## YERSINIOSIS

145

Gastroenteritis = mesenteric involvement

*Y. enterocolitica* M/c

*Y. pseudotuberculosis* M/c mimics appendicitis.

M/c Bacteria  $\hat{=}$  mimics appendicitis  $\Rightarrow$  *Pasteurella multocida*.

$\Delta$  of yersinosis -

Stool culture on buffer saline. Q.

'cold enrichment'

Selective Media  $\rightarrow$  CIN (Cefsulodin Irgason  
Novobiocin media)

CALYMMATOBACTER (KLEBSIELLA) GRANULOMATOSIS  
Intracellular

- Donovanosis / Pseudobubo / Pseudoelephantiasis.

[no lymphadenopathy]

- Safety pin appearing Bacilli in mononuclear cell (PUND cells)  $\rightarrow$  Diagnostic

H. DUCREYI

$\downarrow$   
Pain.

Chancroid  $\rightarrow$  Painful

Requires only Factor X for growth.

A School of fish or Tramtrack or Rail Road

Extracellular

Gram -ve Coccobacilli

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## BURKHOLDARIA PSEUDOMALLEI

shows safety Pin Appearance

Gram -ve.

### Safety Pin Appearance

*Vibrio parahaemolyticus*

Pasteurville

*Burkholderia pseudomallei*

*Calymmatobacter*

*Yersinia*

## FERMENTORS

### VIBRIO

#### VIBRIO CHOLERAE

Comma shaped - single Polar Flagella.

#### Virulence

→ Endotoxin - no virulence

→ Exotoxin

CT (Cholera-toxin) Most Imp

ACE (Accessory Cholera exotoxin)

ZOT (Zona occludens toxin)

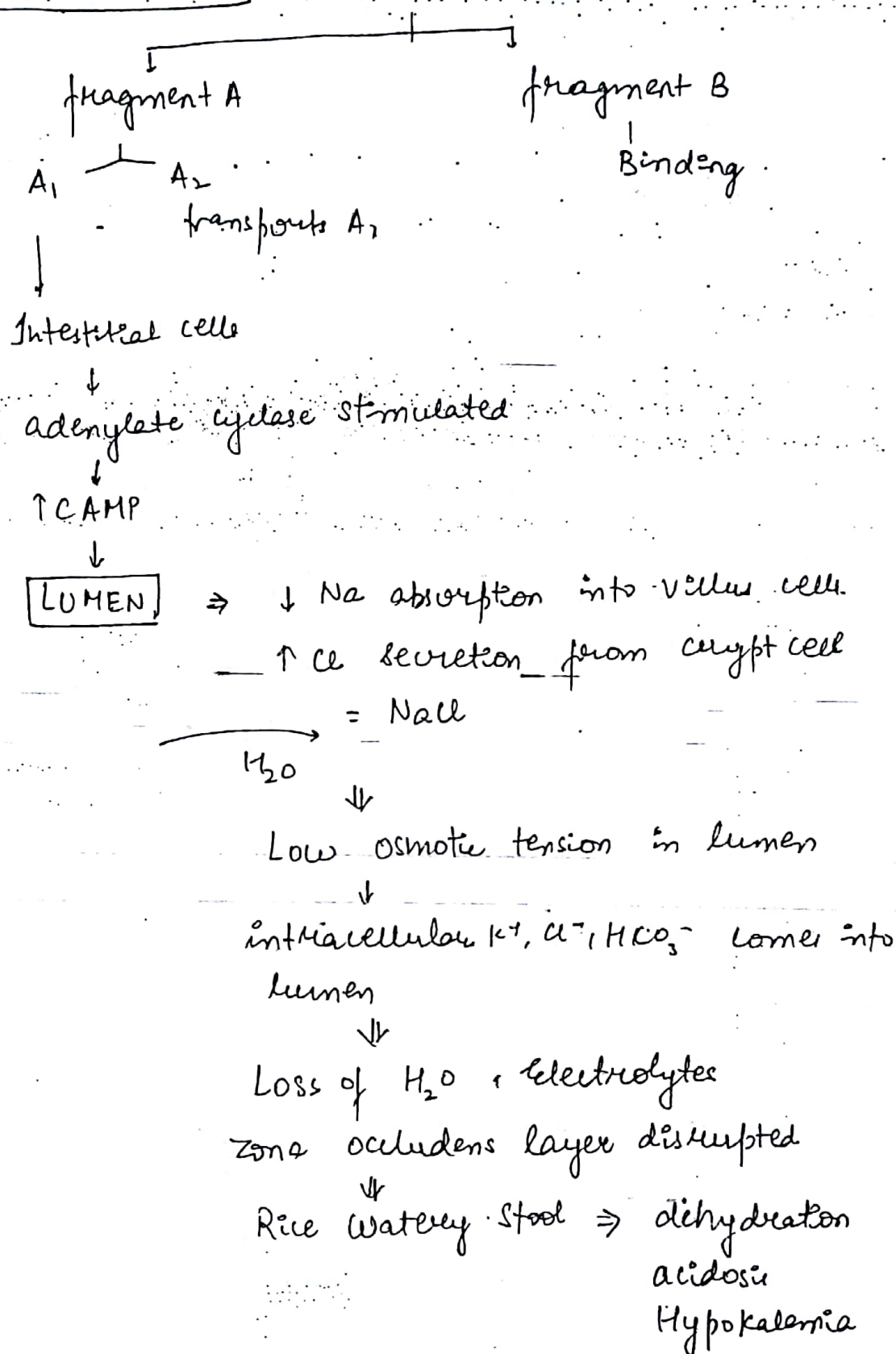
TCP (Toxin coregulated pilus)

found in  $O_1, O_{139}$



# Cholera Toxin

147



## Diagnosis

① Stool Culture

② Enrichment Media → Alkaline Peptone Water  
Monsieur Tauxocholate broth

① Transport Media - Cary Blair

VR media

autoclaved sea water

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② Selective Media - Bile Salt Agar

GTTA (Gelatin Taurate Tellurite)

TcBS (Thiosulphate Citrate Bile Salt  
Substrate)

*V. cholera* → yellow colonies

*Vibrio* → Green colonies. ~~Halophilic~~ [Halophilic].

1) *V. parahaemolyticus* → causes necrotizing enterocolitis

Kanagawa phenomenon ← [β hemolysis on High salt Agar  
containing Blood Agar Media  
[Wagatsuma Media]

2) *V. vulnificans*.

Darting Motility  
other tests -

• String Test

• Oxidase (+)

• Cholera Red Reaction (+)

↳ Nitroso indole formed.

\* Epidemic Period → Source is Human

\* Interepidemic Period → Source is crustaceans in sea water

## 149

```

graph TD
    A(( )) --> B[0_1]
    A --> C[0_139]
    B --> D[Classical]
    D --- E[Severe]
    C --> F[Error]
    F --- G[mild]
    H[Non 0_1]
  
```

7<sup>th</sup> pandemic  
from Indonesia

$\ominus$                        $\oplus$

$\ominus$   $\oplus$

(S) (R)

IV V

~~Biswara~~ Biovars. - clonal & hetero

$$\begin{array}{c} \text{EtO} \\ \diagdown \quad | \quad \diagup \\ \text{O} \quad \text{I} \quad \text{H} \end{array}$$

1st isolated in Chennai

Bengal strain → 1992-94 outbreak in Bengal

It caused outbreak.

So, Non-pandemic strain.

150

O<sub>139</sub> is antigenically, epidemiologically distinct from O<sub>1</sub>

Clinically similar

No cross immunity seen.

### Vaccine

Killed vaccine oral vaccine - classical, El Tor -  
ogawa, Inaba

↓  
↑ Mucosal Immunity

'Copies ~~Ab~~' Q.  
Ab

in stool.

### NON-FERMENTORS

#### PSEUDOMONAS

[Obligate Aerobe]

→ Environment

Colonies have shiny "Gun Metal" appearance.  
fruity odour / Grape odour

NLF, Oxidase +ve

Grows at 42°C

Pigments :-

Pyocyanin - blue.

Pyoverdine - green

Pyorubins - red

Pyomelanin - black



## Virulence Factors

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- ① Pili - Adhesion to cell
- ② Flagella - Adhesion, motility, inflammation
- ③ Capsule - Biofilm
- ④ LPS - Bind to CFTR for internalisation  
Anti-phagocytosis  
Inflammation
- ⑤ Type III Secretion System - ExoS  
ExoU  
ExoT  
ExoY  
Antiphagocytosis
- ⑥ Type II Secretion system - ExoA - inhibition of EF2  
Cytotoxicity of phagocyte
- ⑦ Phospholipase - Cytotoxicity of Phagocyte
- ⑧ Proteases - Proteolytic activity

## Complement System Evasion by Pseudomonas

Elastase & alkaline phosphatase



degrade C1q, C3b/C5b

Δ - Cetrimide Agar - Selective Media

# Typing - Bacteriocin Typing

[Image]

Difference antimicrobial Agents secreted.<sup>152</sup>  
by different strains

Shigella Sonnei - colicin  
Klebsella - Klebocin



Grow strains in between  
1 Remove it next day

↓  
Chloroform application.

↓ standard  
Now put again strain.

↓  
Growth differs due to  
antimicrobial substance released.

Rx

1) Neutropenic host or HDR

↓  
Cefipime

2) Non-neutropenic host

↓  
Monotherapy      Combination

↓  
Ceftazidime

↓  
Pip/taz or

Carbapenem or  
Ampicillin

## BURKHOLDARIA PSEUDOMALLAI

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- ④ Capsulated
- ⑤ Most virulent amongst Non-fermenters.  
It has all ~~same~~ skeleton system + capsule.

⑥ ~~Mold~~ Melioidosis → Pulmonary Infection  
Abscess

pneumonia  
necrotizing lung Disease  
skin ulceration  
Lymphadenopathy

④ Bioterrorism - Cat II agent

⑤ Long Latent Period → 'Vietnam Time Bomb Disease'

⑥ Δ → Gram -ve  
Safety Pen

Culture - Ashdown media -  
cornflower head wrinkled  
Purple colonies

Doc - Meropenam / TMP-SMX.

## STREPTOTROPHOMAS MALTOPHILIA

(Non-Fermenter)

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Gram -ve,

Motile (polar)

Environmental

HAI

Δ - culture ✓ Lavender Green

✓ Gray colour colonies on blood agar

oxidase ⊖

Oxidation of glucose + maltose

Doc- TMP-SMX \* Ticarcillin - clavulanic acid

## ACINETOBACTER

Environmental, HAI

Non-motile

oxidase ⊖

R<sub>x</sub> - same as pseudomonas.

Non-Fermenter (Zoonotic)

## BURKHOLDARIA MALLEI

Equinus (Horse) → Glanders

## BURKHOLDARIA CEPACIA

causes Cepacia Syndrome

associated w/ sepsis

Δ - Colistin containing ~~media~~ agar.



Pneumonia in Cystic Fibrosis = mucoid colonies

↳ B. cepacia

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## BACTERIAL VAGINOSIS

caused by: *Gardnerella*

*Bacteroides*

*Mobiluncus* sp.

↓  
— *Lactobacillus*

Nugent score  $\geq 7 \Rightarrow$  Diagnostic

Based on less no. of *Lactobacillus* & more pathogens.

Clue cell - Epithelial cells studded = Gram -ve bacteria

Whiff test - Amine (fishy odour)

pH  $> 4.4$ .

## CHROMOBACTERIUM

## VIOLACEUM

Purple coloured.

Lead to lung & wound infection

Gram -ve

## SERRATIA Marcescans

*Pseudomonas*

## CAPNOCYTOPHAGA

156

Slow growing

Capnophile

Gram.-ve

- Fusiform. filamentous

Gliding Motility

Modifies chemotactic ~~factor~~ activity of neutrophils

Fulminant Infe<sup>n</sup> in asplenia or alcoholism.



Fusiform  
Gram.-ve

## HACEK

Haemophilus parainfluenzae (Hc) sp. isolated from.  
- HACEK endocarditis

A. actinomycetemcomitans - Prosthetic valve  
endocarditis.

Cardiobacterium Homini → aortic valve

Eikenella corrodens → Least common cause of HACEK.  
endocarditis

Human Bite → clenched fist injuries Q.

Kingella Kingae - septic arthritis in children. (H/d)  
Purpuric rash similar to meningococcus

# LEGIONELLA PNEUMOPHILIA

BCYE

157

\* Charcoal in the ~~BYCE~~ media used to isolate Legionella pneumophila

↳ (Buffered charcoal Yeast Extract)

↓  
detoxifying agent

[Black colonies]

\* Contaminated water (amobas/algae)

↳ aerosol through A/c  
aspiration.

→ No person to person transmission

Legionnaires Disease  
Pneumonia

Pontiac Fever  
No pneumonia

Δ - ① Neutropenia

② Culture on BCYE media [charcoal for detoxification]

↓  
Monoxenic media.

(Requires 1 biological agent in media)

③ used in parasite cultures

③ Immunofluorescence - sensitive method

Pfeiffer's Bacillus

aerobe

oxidase ⊕

pleomorphic

resp. sample - coccobacilli

CSF - filamentous.

1st organism - entire genome sequenced

2 months to 3 years - No Ab to PRP

Biotype 1 is M/c cause of meningitis

Virulence

capsule → 6 serotypes (a to f)

Type b → capsule has PRP  
[95% infection] (polysaccharide ribitol PO<sub>4</sub>)

↓  
causes invasion.

5% of infection - Non-capsulated or  
Non-Typable strains

Vaccine - Hib

(monovalent polysaccharide conjugate vaccine)



Δ - Require X, V factor for growth.

159

① Culture

a) chocolate agar - factor V released

b) Blood agar - S. aureus

c) Nutrient agar - disc of X, V factor

d) Levinthal agar (Capulated strains produce incidence)

e) Fildes Agar - peptic digest of blood in nutrient agar.  
(Best)

— Satellitism in H. influenzae is due to factor V.

~~Colon~~ Bigger colony around S. Aureus.

↓  
It stimulates production of V factor

### H. AEGYPTICUS

Koch's weeks Bacilli or Brazilian purple fever  
Egyptian conjunctivitis Red eyes.

# BORDETELLA PERTUSSIS (Gram -ve coccobacilli)

Whooping cough → Inspiratory stridor

160

100 day fever

No animal reservoir unlike B. Bronchoseptica

Virulence -

① capsule → No Role in Virulence

② Pertussis Toxin (Type I, IV) - ~~Complete~~ secretory system  
activate adenylate cyclase → ↑ cAMP

③ Tracheal cytotoxin

④ Adenylate cyclase Toxin

⑤ Dermonecrotic toxin

⑥ Endotoxin

⑧ Adhesin (Type V) → FHA, pertactin, fimbriae  
secretory system BAKA protein

↓↓

↑ Histamine

↑ insulin

Lymphocytosis → huge

△ ① Thumb Print appearance

② Regan Lowe → Mercury Drops or Busted Pearls

(Charcoal + 10% horse blood + ceftalexin) Regan Lowe ⇒ Transport media

③ Bordet Gengou

Vaccine

Whole celled

→ Potato Blood Agar

④ Ab detect by ELISA

⑤ PCR 15461 - PT promoter gene

contains ↓ more safe

PT - pertussis toxin

FHA1 - fimbriae

FHA2 } filamentous

FHA3 } haemagglutinin Ag

Pertactin.

This menet - preservative

✓ FRANSICELLA

TULARENSIS

Zoonotic

- Transmission - ticks / deer fly or direct contact c rabbits / mink rats
- penetration of skin M/c inhalation, ingestion

- Culture media

✓ chocolate agar

✓ MTM (mod. Thayer Martin)

✓ BCYE

Gram -ve Cocci bacilli

Δ - Serology

(M/c)

R<sub>x</sub> - resistance to  $\beta$  lactams

162

streptomycin / gentamicin

tetracycline

chloramphenicol

FQs

CAT A agent of Bioterrorism.

(BSL III) req for culture.

M. TB

① Obligate aerobe

② Micro-aerophilic  $\rightarrow$  M. Bovis.

③ Virulence factor

- CORD factor.

$\Delta \rightarrow$  M/E  $\rightarrow$   $\downarrow$  sensitivity

$10^4$  bacilli/ml of sputum is req.

a) Zn stain - slender, long, curved.  
[for confirmation] beaded

b) Auramine Rhodamine - Direct fluorescence assay

$\downarrow$  Binds to mycolic acid

Highly sensitive  $\rightarrow$  used for screening



## 27 Culture

LJ media

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Egg containing media = asparagine  
Malachite Green (Selective)

M. Tb → Eugonic growth

M. Bovu → Dysgonic growth (sparse)

Petroff's Method → sputum processing.  
(15-20 min)

↓  
4% NaOH  
N-Acetyl Cysteine (mucolytic agent)

↓  
Centrifugation at 1 RPM

⇓  
BSL III (Biosafety Level)

✓ Best  
CBNAAT (Cartridge Based Nucleic Acid Amplification Test)

- PCR

- BSL - II

INNO LIPA (Line probe assay)

Reverse Hybridisation technique.

20 DNA probes of different Mycobacterial species  
are immobilised on nitrocellulose strip. the  
amplicons (amplified DNA) are hybridised

to the probes

Chromogenic Reagent is used instead of radioactivity

GTMD (Genotype Mycobacteria Direct)

NASBA (NA. where RNA is amplified & reverse <sup>16S</sup> hybridised = RNA probe on strip.  
Nucleic acid sequence based assay  
Only 5 species detected

IS6110 - Best for Genotyping

Best Culture Technique - BACTEC NGIT.  
↓  
✓ fluorescent  
✓ 2 days - 7 days

Latent TB

Mantoux Test → 5 TU of PPD-S  
or 1 TU of RT<sub>23</sub> strain ← RNTCP  
after 72 hrs ↓  
in L forearm.

>10mm induration along short axis  
on L forearm

⇓  
Positive → exposed

Quantiferon Gold Test / IFN-γ Release Assay IGRA

Whole blood  
↓  
Stimulate = M.Tb Ag. eg. -  
Lymphocyte → IFN-γ  
E.CAT.6  
CFP 10  
M.Tb 7.7  
⊕  
exposed

⇒ less false +ve

Q No. 1000 Rx = BCG or atypical Mycobacteria  
or NTM or MOTT  
except M. Kansasi.

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## ATYPICAL MYCOBACTERIUM

Runyan classification

Photochromogenic → Pigments in Light

M. Kansasi

M. Maxium [swimming pool / Fish tank granuloma]

Scotochromogenic → Pigments in dark

M. Scrofulaceum

M. Szulgai

[crow gar in dark]

Non-Photochromogenic - No pigments.

M. ulcerans → Buruli Ulcer

M. Avium → < 50 CD4 cells in HIV

M. Avium

M. intercellulari / (Batey Bacilli)

Rapid Growers -  
(< 7 days)

M. Chelonae

M. fortuitum

} → cause post trauma abscess

[loan + fortune = rapid growers]



MTB

Atypical

166

① Niacin (+)

(-)

② ~~Amo~~ Amyl Sulphatase (-)

(+)

M. LEPRAE [acid fast staining w/ 5% H<sub>2</sub>SO<sub>4</sub>]

SSS (short, stout, straight bacilli)

→ [Image]

- acid fast B. in palisade

750 bacilli - globi

in histiocytes - FOAM cells

specimen

SSS - split skin smear

(6 specimen → 4 skin, 1 ear lobe, 1 nasal mucosa)  
+ nt in edge of lesion

Ab to PGL1

Δ

M/E  
IOC

- ↓ sensitivity

10<sup>4</sup> bacilli / gm of tissue.

culture - Best Armadillo (natural infection)

M/c - Mice (suckling)

↓

Thymectomy

(to induce lepromatous leprosy)

LEPTOSPIRA

Leptospira → ?

Dark Ground Microscopy

←

Treponema

Light microscopy → Borrelia



## SPIROCHAETES

Spiral organisms & endoflagella → cork screw <sup>167</sup> motility

T. Pallidum → venereal Syphilis

1<sup>st</sup> stage → Hunterian chancre

↓  
painless, indurated.

self-limiting in 4-6 weeks.

2<sup>nd</sup> → Dissemination.

after 2 to 6 months.

↓

Condyloma

Latent → No sign & symptom

3<sup>rd</sup> → complications.

Aortic aneurysm  
Tabes Dorsalis

Δ -

1<sup>st</sup> stage → ① Dark Ground Microscopy

↓  
only reflected light ~~through~~ from object  
through special condenser

Resolution power not increased.  
only optical illumination used.

② EIA (Enzyme Immuno Assay)  
IOL

2° stage → TPPA (Treponema Pallidum Particulate Agglutination)  
(Easy) <sup>gelatin</sup>  
✓ Most sensitive, specific. Overall  
Used for Confirmation.

Screening → VDRL  
slide flocculation Test

↓  
Cardiolipin Ag [Calcutta Ag]  
(derived from ox heart)

Reactive → clumps.

[Image]

Non-reactive → Scattered

Using microscope → floccule seen.

RPR

Cardiolipin + carbon particles  
Particle agglutination based test

Nichol Strain → Pathogenic Treponemes

TPI

not safe but ↑ specificity

TPHA - haemagglutination assay

↓ sensitivity

FTA-ABS

Reiter's Strain

→ non-pathogenic treponemes

(Difficult)

✓ Absorption

then

Non-specific Ab in serum

Nichol's Strain  
used

[Indirect Immunofluorescence]

## LEPTOSPIRA

169

- ① Curved at 1 end.
- ② M/c Zoonotic disease.

? interrogans.

- ③ 3R → Rats, Rainfall, ricefield  
Rat urine → penetration of skin.

affect liver & kidneys

Jaundice haemorrhage fever & myalgia

## WEIL'S DS / Andaman Fever

1 ① Culture on Korthoff, Fletcher, EMJH media

② Ag detect<sup>n</sup> in urine

③ Typing → Serum is used.  
(Leptospira difficult to grow)

Reference Lab

Macroscopic agglutination Test (MAC)

↳ Killed Leptospira Ag used to detect  
Serogroups  
↓  
Serotypes having similar Ag

Passive Agglutination

Serogroups detected.

Microscopic agglutination test → Serotypes detected

Live Leptospira

immobilisation test → Dark Ground  
Microscope used

# BORRELIA

170

Thick → can be seen in Light microscope

Causes Relapsing Fever

Epidemic

Louse - B recurrente

Endemic

Tick born

B. Burgdorferi → LYME'S Disease

Erythema chronicum Migrans

[Image].

↓  
Concentric Centrifugal Rash

Δ

P66 Ab by western Blot Assay

(Non-specific But Best)

25/2/18

## RICKETTSIACEAE FAMILY

→ Rickettsia } Weil ~~Fle~~ Felix Test

→ Orienta

→ Coxiella

→ Ehrlich

↓  
Heterophile Agglutination Test

↓  
Non-motile Proteins strains

P. vulgaris

Ox 19

Ox 2

P. mirabilis

OxK.

Russia in endemic for Typhus Fever



# TYPHUS FEVER

Epidemic

Louse

causative

R. Prowazeki

Endemic

Flea

causative

R. Typhi

Brill Zinsser<sup>171</sup>

(Reverberant)

P. Prowazeki

Some of the elderly not suffering from Disease

§ Spotted Fever

Tick Borne

Ox2. - Rocky Mountain - R. Rickettsiae  
Fever

Indian tick typhus

✓ Japanese " "

✓ Siberian " "

R. conorii

mite → Rickettsial pox → R. Akari Q

Scrub Typhus

Oxk

Thrombiculid

mite (Larva)

→ Orientia

tsutsugamushi

Epidemic Typhus

Endemic Typhus

✓  
Neil Moseley or

Tunica vaginalis  
test

⊖ in

R. Prowazeki

⊕ in

R. Typhi

CV  
AV  
TV

## ✓ Q FEVER / ABBATOIR / QUEENSLAND FEVER

→ Tick act as reservoir → transmit to sheep, goat, cattle<sup>172</sup>  
but not to humans

→ Transmission to humans by respiratory route -  
aerosol from soil, tissue or during parturition.  
only rickettsial infect<sup>n</sup> can be transmitted out arthropod i.e.  
man to man → resp. route

→ Acute Infection → Influenza like

→ Chronic " → Infective Endocarditis M/c

→ Risk Factors - Pre existing valve anomaly  
- Immunocompromised

Δ -  
→ Culture only in cell lines [BSL 3 lab]

- Δ Immunofluorescence 10c  
Giemsa stain.

PCR

≡ Coxiella Survival Holder's Method by pasteurization.

↓  
(63°C) for 30 min.

[Flash method - (72°C) for 13-15 sec].

Ehrlichia → Morula (vacuole c<sup>n</sup> phagocytes)

sp  
Sennetsu

→ raw fish.

Chaffensis

}- tick.

→ affect monocyte

Phagocytophila

→ affect granulocytes.

# CHLAMYDIA

Gram -ve cell wall But no peptidoglycan. (no neuraminic acid)

Lack ATP synthesis  $\rightarrow$  Energy Parasites

Infectious form k/n/a Elementary Body  
(50-330nm)

$\Delta$  form  $\Rightarrow$  Reticulate body

Pathogenicity :-

C. Trachomatis  $\rightarrow$  H.P. Body (Heidraden Prowazeki)

serotype A-C  $\rightarrow$  Trachoma

D-K  $\rightarrow$  Inclusion Conjunctivitis  
NGU

$\rightarrow$  Fitz Hugh Curtis  
 $\rightarrow$  Reiter's Syndrome  
conjunc  $\rightarrow$  Poly arthri-  
urethritis

LGV<sub>1+3</sub>  $\rightarrow$  Lymphogranuloma Venereum  
 $\rightarrow$  painless ulcer, painful bubo

$\Delta$  - TRIC Serovars

M/E  $\rightarrow$  Giemsa, Gimenez, Fastenede;  
Machiarella.

LGV  $\rightarrow$  Miyagawa corpuscle

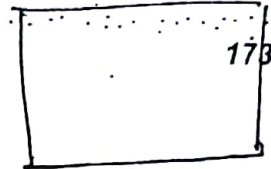
$\rightarrow$  culture on MacLoy cell lines

$\rightarrow$  Ab detection by ELISA

$\rightarrow$  NAAT by PCR.

LGV Serovars  $\rightarrow$  Fluor Test

Ab detect by Micro Immunofluorescence  
Assay



① Inclusion Bodies  
(Reticulate Body)

② MacLoy Cell Lines



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↳ LPS → Genus Specific Ag (CF Test)

3) Outer Membrane protein → serovar or serotype specific Ag [Micro IF test].

My fried egg glided in  
dining while eating

M/c cause of atypical pneumoniae or walking pneumonia

✓ Steroid +nt in cell-membrane

No turbidity in liquid media → also seen in B. anthracis.

① Culture in media  $\bar{c}$  cholesterol

✓ Diene stain of isolates [Methylene Blue + Azure]  
fused egg colony

② Serology → Heterophile agglutination Test

a) Cold agglutination → 'O' -ve RBC at 4°C.

~~by Streptococcal MG Test~~



## HELICOBACTER

- 1) Deep in mucous layer on epithelial side (pH - 7.4) 175
- 2) ~~Protects~~ <sup>Proteases</sup> → modifies gastric mucosa - reduce diffusion of acid.
- 3) Urease activity → Ammonia - buffering of acid
- 4) Complement system evasion by H. Pylori  
Protectin inhibits MAC → C5-C2

Δ

1) Warthin Starry Silver stain → Sea gull wings.

↓  
also used for

Campylobacter

microaerophilic

✓✓ - comma shaped  
darting motility  
↓

Microaerophilic (5% O<sub>2</sub>)

media:- Skirrow's  
for Both. Butzler's

HLA: B27 - Reactive arthritis.  
Trigger guillian Barre syndrome  
(serotype O19)

CAMPY Blood agar. grows at 42°C  
gullwing shaped, darting motility

Pathogenicity:-

Helicobacter → peptic ulcers

↓  
Adenoma / Malignoma

Campylobacter → Gastroenteritis & blood.

↓  
Reservoir (poultry)

Gold std. for Δ of Helicobacter → Urea Breath Test

Biopsy & urease → for dysplasia

# BARTONELLA BACILLIFORMIS

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## CARRION'S DISEASE

Oraya Fever - Acute phase

← [Female Lutzomyia]  
(sandfly)

- fever, other constitutional symptoms
- severe anaemia, jaundice, Hepatomegaly
- lymphadenopathy, myalgia

Verruca Peruviana - after resolution of oraya fever  
[Image] Non tender,  
red to purple  
nodular lesion.

Δ:-

M/E → Warthin Starry or Giemsa stain

⇒ Bacillin in RBC

Culture on blood agar at 30°C

Ab detect

PCR.

B. QUINTANA →

French fever (5 day fever)

By Louis

caused outbreak

B. HENSELAE → Cat Scratch Disease → cervical LN

Bacillary Angiomatosis

Cat Bite → Pasteurella

## RAT BITE FEVER

177

Spirillum Minor

3-5  $\mu$ m

rigid spiral org.

Rat bite fever (Sodoku)

Local lesion, regional gland.

swelling, skin rashes.

Culture - inoculation in guinea  
pig / mic

Flagella at Both end

Streptobacillus moniliformis

Gram (-ve)

Aerobic

Pleomorphic bacteria that  
forms irregular chains of bacilli  
interspersed with fusiform  
enlargements

L-forms seen

Rat bite fever - Septic fever  
Blotchy

— Petechial rash —  
painful Polyarthritides

Ingestion of milk -

epidemics of Haverhill  
fever

Culture - trypticase soy  
enriched with 20% blood.

## BRUCELLA

Zoonotic

(from animal milk)

affects the reticulo-endothelial system

Intracellular

— Hepatosplenomegaly

— Lymphadenopathy

Malaria or undulating fever (typhoid like illness)

— Neutropenia

Triad

- 1) undulating fever  
fever with night sweats
- 2) arthralgia
- 3) hepatosplenomegaly



DOC - Doxycycline + streptomycin.

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Δ - <sup>90</sup>Casteneda Blood culture.  
'bypathie medea'

Serology

→ coombs test

→ std. agglutination test

11 tube dilution.

"PROZONE" Phenomenon.

→ PCR. → Gold std

Silver Impregnation → Levadite / Fontana  
for spirochaetes.



# PARASITOLOGY

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Protozoa  
(unicellular)

Helminths  
(multicellular)

Platyhelminths  
(flat)

Nemathelminths  
Round.

**Cystodes**

(segmented)

**Trematodes**

(unsegmented)

**Nematodes**

Digestive, excretory  
& reproductive spaces  
are separate

Incomplete  
alimentary canal

complete  
alimentary  
canal

**NO CYCLOPS**

"NO alimentary canal"

Hermaphrodites except  
schistosoma

Separate sexes

## INTESTINAL PROTOZOA

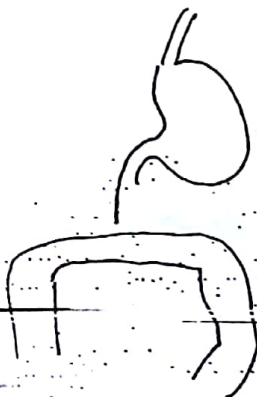
ENTAMOEB HISTOLYTICA (pathogen)

[Image]

Infective form  $\Rightarrow$  Quadrinucleate Cyst

Excystation occurs in ileum ( $\uparrow$  pH)

cyst  $\rightarrow$  Trophozoites



M/c site - sigmoid colon  
caecum (M/c)

## Virulence factors:-

- ① Galactosamine Lactin → binding
- ② Protease → degrade collagen
- ③ Calmodulin phosphatase A } inhibit phagocytosis
- ④ Thioredoxin Reductase → degrades toxic  $O^{\cdot-}$  &  $NO^{\cdot}$  radicals.

At Distal colon → encystation occurs.  
Trophozoites → cyst

## Diagnosis:-

① M/E of stool. (wet or iodine or trichrome stain)

<u>Pathogens</u>		<u>Non-Pathogen</u>	
<u>Trophozoite</u>	15-20 $\mu m$ active	20-30 $\mu m$ sluggish	
	RBC or cellular debris in trophozoites [erythrophagocytosis]	Bacteria in trophozoites	
<u>Nucleus</u>	central Ectoplasm + Endoplasm [thin] [granular]	eccentric Endoplasm	
<u>Cyst</u>	6-15 $\mu m$ 1-4 nuclei	15-20 $\mu m$ 1-8 nuclei	
<u>Glycogen Mass</u>	uninucleate stage	Binucleate stage	
<u>Chromatoid Body</u>			

No difference Q.

Δ Rectal Biopsy  $\Rightarrow$  Flask shaped ulcer.



Other ways of A

① Nested PCR

② zymodene assay  $\rightarrow$  isoenzyme pattern on electrophoresis

③ galactosamine Lectin Ag detection by ELISA ] IOC for  
Gal / Gal NAC ELISA ] Invasive amoebiasis

## EXTRAI NTESTINAL AMOEBIASIS

~~Ham~~ Hematogenous Route

Liver (M/C)

Lung

Brain

—  $\rightarrow$  Posterosuperior quadrant of R lobe

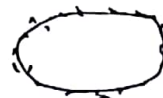
Cutaneous (directly)

$\rightarrow$  cutaneous amoebiasis.

Δ

① M/E of Liver aspirate  $\Rightarrow$  Anchovy sauce pus  
trophozoites  $\downarrow$

② PCR  $\rightarrow$  most sensitive



Trophozoite is  
at periphery

③ Radiology

④ Clinical diagnosis

## FREE LIVING AMOEBAS

Found in natural water source

Acanthamoeba  $\rightarrow$  1<sup>o</sup> lung infection

haematogenous

Granulomatous Amoebic  
encephalitis.



Keratitis (contact lens)

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$\Delta$  - CSF  $\Rightarrow$  Both Cyst + Trophozoite

Naegleria  $\rightarrow$  flagellated amoeba

$\downarrow$   
travels via olfactory n/v

$\downarrow$   
pierces cribriform plate

$\downarrow$   
1° amoebic meningoencephalitis

$\downarrow$   
fatal

$\Delta \rightarrow$  CSF only trophozoites

- A of free living Amoeba

$\rightarrow$  culture on non nutrient agar  $\bar{c}$  E. coli  $\rightarrow$  Tracts.

$\rightarrow$  M/E of CSF

• H. E. stain  $\left[ \begin{array}{l} \text{Cyst + trophozoite} \rightarrow \text{acanthamoeba} \\ \text{Trophozoite} \rightarrow \text{Naegleria} \end{array} \right.$

• Immunofluorescence [Image]

## GIARDIA INTESTINALIS

Infective form - Quadrinucleate Cyst

Excystation takes place at the Jejunum - M/c site

Trophozoites bind  $\bar{c}$  ventral sucking disc

$\downarrow$   
disrupt brush border enzymes

$\downarrow$   
Malabsorption

Steatorrhea

foul smelling stool

Anaemia

( $\bar{c}$  IF)  $\left\{ \begin{array}{l} \text{Megablastic} \\ \text{Pernicious} \end{array} \right.$



## Diarrhoea

Stool → "Loose Greasy" yellow colour

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Δ -

- ① Stool M/E for trophozoites + cyst (M/c)  
wet mount, trichrome, Kohn, Giemsa
- ② Direct Immunofluorescence assay → auramine
- ③ Ag Detection by ELISA + rapid immunochromatographic assay
- ④ Other test - Antero test → string ingestion + then exam.  
Duodenal Biopsy.  
NAAT

Each Trophozoite has 4 pairs of flagella [Image]

→ [1 ant  
→ 1 middle  
→ 2 post.]

## BALANTIDIUM COLI

Ciliated.

↳ LARGEST PROTOZOA

Infects Pig, humans (occasionally)

Asymptomatic (M/c)

Persistent Intermittent Diarrhoea.

Doc - Tetraacycline

Δ - stool M/E → trophozoites (cysts rarely seen)

Tissue staining ± H&E after endoscopy

[Differential Interference Contrast → for cilia]

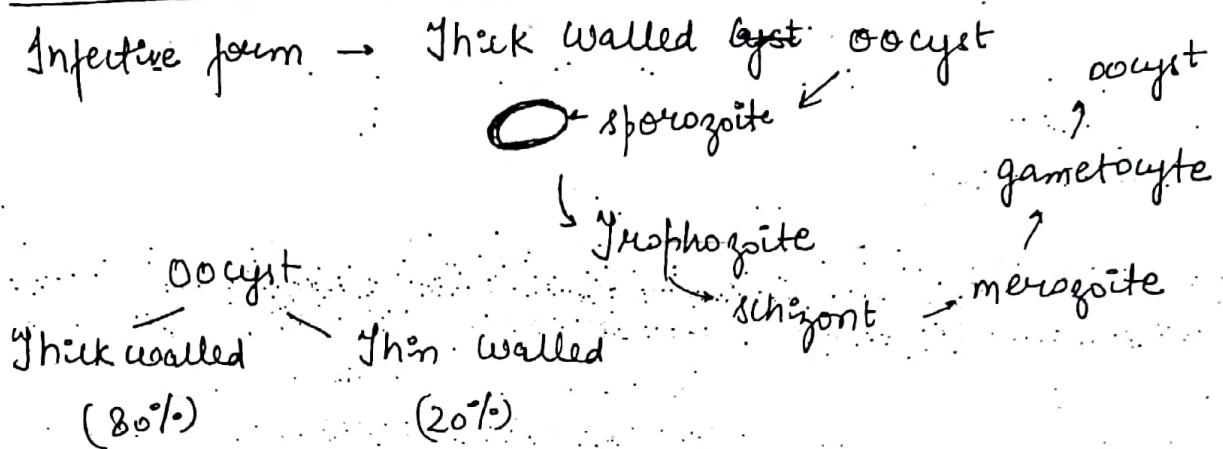
# COCCIDIAN INTESTINAL PROTOZOA

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Diarrhoea in HIV pt + extremes of age

## CRYPTOSPORIDIUM PARVUM (6µm)

Infective form → Thick walled cyst oocyst



No t/t, only improve immune system Persistent Diarrhoea

## CYCLOSPORA (10µm)

autofluorescence. seen.

## ISOSPORA

① Kinyoun stain [Modified Ziehl-Neelsen stain] ⇒ Acid fast oocyst  
Δ :- ~~Kinyoun~~ stain cold technique

Cryptospora - 6µm
Cyclospora - 10µm
Isospora - 25-40µm

A Decolorizing Agent → 0.5% H<sub>2</sub>SO<sub>4</sub>

\* ~~Ghost cells~~ → unstained cells

## ② Immunofluorescence Assay by auramine

185

### MICROSPORIDIUM

↳ earlier considered coccidia

- Fungi
- cause diarrhoea + ocular infec<sup>n</sup>
- spores seen
- Δ :- "Weber stain"

### PLASMODIUM

Pigments

- ?

Peripheral Blood Smear - Gold Std

P. vivax

Δ :-

RBC

Enlarged + Pale

Schuffner's - yellow

Single infection

~~1 RBC~~

1 RBC = one parasite

All stages

P. Falciparum

Normal

Maurer's dot - Black

Multiple infec<sup>n</sup>

1 RBC - multiple parasite

Early + Late stages

↓

SEQUESTRATION

↓

①

Vascular endothelial cells

Mech: of

a) Cytoadherence.

- adhesion molecule

b) Rosette formation

- 2-3 parasite stick together

c) Agglutination - surface adhesion on RBC



P. vivax



Ring form

Romanowskii stain

[Early Trophozoite]

Amoeboid form

[Late Trophozoite]



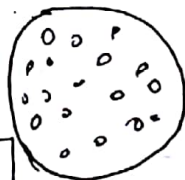
Schizont



completely fills the RBC

Merozoites

> 12 in no.



Gametocyte

P. Falciparum

causes

Renal failure



acule forms

'signet Ring'

Divided Nuclei is also seen here

Crescent or Sausage



Gametocyte

P. MALARIAE

→ causes Nephrotic Syndrome

Zeimann's Dots  
(light Brown)

RBC - normal

Band forms



Basket forms



Merozoites



≤ 8 No.



P. OVALE

Similar to vivax

James Dot's

Dark Brown

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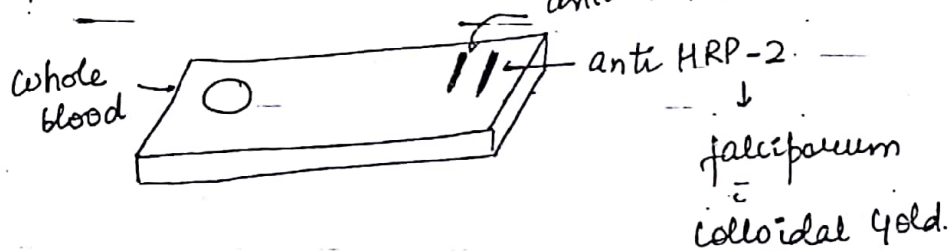
Quotidian → Shinton + Mulligan

Thick Smear → Screening

Thin Smear → Species identification

Other tests for Diagnosis

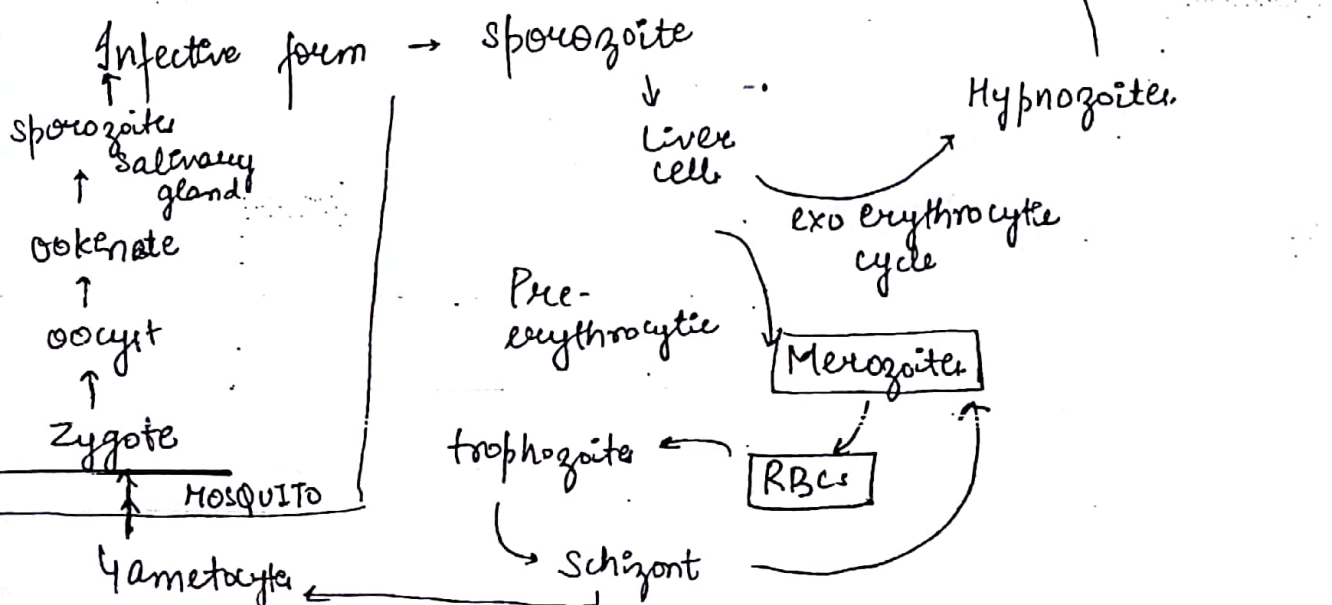
1) Rapid Immunochromatographic assay



2) Quantitative Buffy Coat Technique

Acridine Orange for fluorescent

LIFE CYCLE



\* Transfusion Malaria / Mother to child.

H/c Trophozoite  
Schizont  
Merozoite

short I.P.

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as No pre-erythrocytic cycle

\* Screening of blood  
Serology

→ Rapid. Immunochromatographic Assay

Advantage of Rapid.

No expertise required.

Falciparum

quinine therapy. - Black Water Fever

↓  
acts as hapten  
quinenised RBC

↓  
autoimmune HSN II  
hemolysis of RBC.



complement mediated

quinine → cause hypoglycemia  
hence should be accompanied  $\approx 25\% D$ .

BABESIA MICROTI - Protozoa invading RBC

Jak Borne

RIF → splenectomy

Maltese cross tetrad. → seen in RBC.  
 $\Delta$  form

# HAEMOFLAGELLATES

Leishmania  
Trypanosoma

## LEISHMANIA

L. Donovanii } → Visceral Leishmaniasis  
L. infantum }  
L. chagasi - new world.

Infective form → Promastigote (flagellated form.)

↓  
deposited on the skin by

Sand fly

Phlebotomus - old world

Lutzomyia - new world

↓  
Penetrate the skin  
through tracks

AMASTIGOTE

Spleen  
95%

BM  
65-80%

LN  
50%

↑↑ IL-10

↓

inhibits TH<sub>1</sub>

↑↑ TH<sub>2</sub> response

↓

IL-6 released

Proliferation

↓  
Massive splenomegaly  
Kalaazar.

Δ ① M/E of Bone Marrow.

Bert  
Test

Macrophage → LD Body

1<sup>st</sup> kinetoplast on amastigotes

↓  
'Dot & Dash' [Image]

② Napier Aldehyde / antimony test  
Serology

③ Montenegro Test  
Negative except in Sudan

④ Culture on NNN medium.

↓  
Rosette formation of promastigotes.



Cutaneous LEISHMANIASIS

L. Tropica M/c

Delhi Boil or Oriental Sore.

Mucocutaneous - L. Braziliensis M/c

L. Mexicana complex

PKDL - Nodular Lesion on face after Visceral Leishmaniasis

↓

East Africa [Sudan] - after 2-6 months

Indian Subcontinent - (Bangladesh)

> 3 years.

India - W.B., Bihar, Assam



## TRY PANOSOMA

T. CRUZI

T. BRUZI

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### T. CRUZI

causes Chagas Disease



Chagoma

Infective form

→ Trypanomastigote

↓  
deposited by

Triatoma / Reduvid / kissing or  
Assassin



chagoma - skin lesion.

cardiomyopathy

megacolon

Romana Sign ⇒ u/L Periorbital edema

Δ - Amastigote in (heart) tissue.

C-shaped Trypomastigote in blood

### T. BRUZI

causes Sleeping Sickness

Infective form → Trypomastigote



vector - Tsetse Fly



inhibition of Insomnia Receptors.

Winter Bottom Sign ⇒ cervical L.V. enlarged

Δ - NO amastigotes.

ELONGATED PROMASTIGOTES

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glycoprotein switching

↓  
Immune evasion.

## TRICHOMONAS VAGINALIS

[Image]

- 1 morphological form →
- 4-5 ant. flagelle + 1 along-undulating body



- Greenish frothy D/c
- Whiff Test +ve

## LMP TOXOPLASMA

A ♀ = H/O recurrent abortions present to ANC in her

1st trimester	TORCH screen	
	↳ IgM.	IgG.
	Toxo +	+
	Rubella -	-
	CMV -	+
	HSV -	+

Next Management ?

a) Start spiramycin

b) advise MTP

c) IgG avidity test

d) IgA detection

## Toxoplasma Gondii

Transmission through cats & canines.

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Infective form  $\Rightarrow$  oocyst in cat faeces

$\downarrow$   
Ingested

Man - accidental host:

$\downarrow$   
Bradyzoites

$\downarrow$   
Tachyzoites (motile)

$\downarrow$   
Tissue cyst (Bradyzoites) (dormant)

Sign, symptoms develop in Immunocompromised,

♀

Reactivation.

HIV pt.

$CD_4 < 50$

Fetal encephalitis

Δ - ① No IgM, low IgG titre

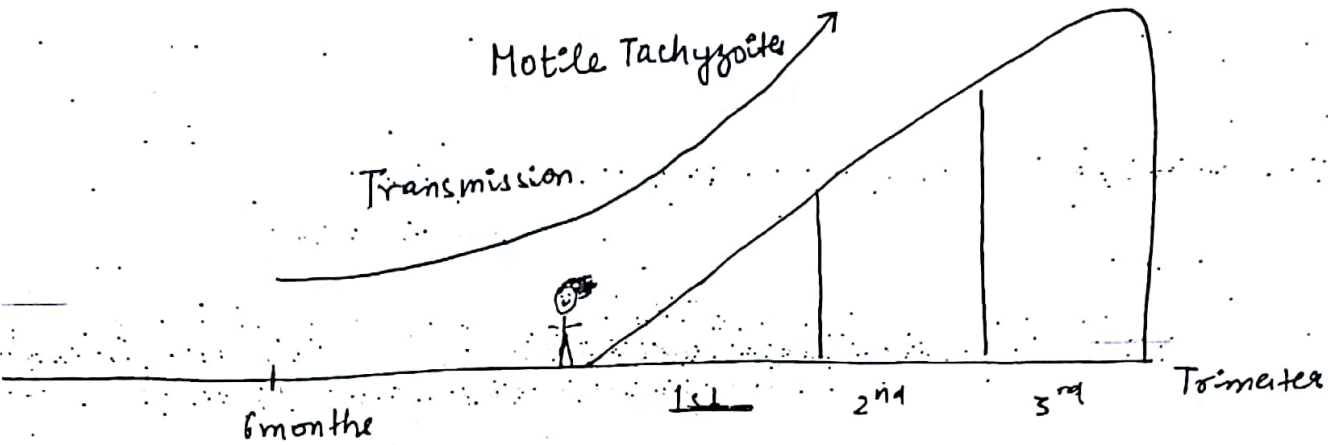
② MRI - Crescent shaped multiple  
Ring enhancing lesion.

Eccentric target sign

③ PCR of CSF.

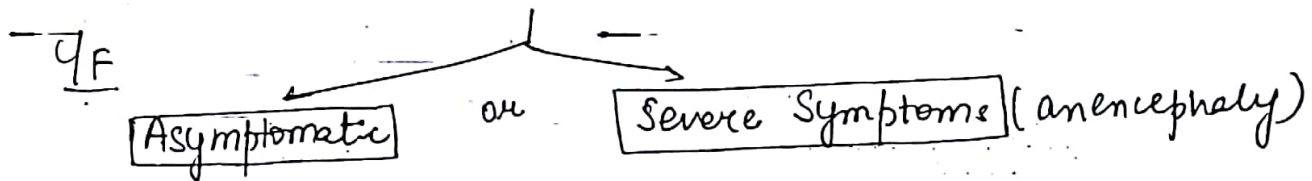
$\downarrow$  spiramycin - DoE

In ♀



Beyond 6-months  
no tachyzoites  
↓  
no transmission

← fetal affection more in earlier part



Late sequelae of Choroio-retinitis

Lead to Blindness

Risk Assessment In Cong. Toxoplasmosis :-

By Serology

IgG +  
IgM -  
↓  
Past Infection  
[Not significant]

IgG +  
IgM + (stays for ≥ 1yr)  
↓  
IgG avidity Test (measures infection affinity)  
↓  
High  
Low

IgG -  
IgM +  
↓  
Recent infection

IgG -  
IgM -  
↓  
Susceptible  
↓  
Preventive



IgA detection Low IgG avidity

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↓  
more sensitive test for recent inf.

Sabin Fieldman dye Test.

to detect Ab

↓  
Not recent Inf.

## CYSTODES

Developmental stage

Eggs - Coracidium - Procercoid Larva - Pleurocercoid Larva

Trematodes Developmental Stage

Eggs - Miracidium - sporocyst - Radiae - Cercariae - Metacercariae

Definitive Host

T. Saginata  
Diphyllobothrium  
Hymenolepis  
Dipylidium.

Intermediate Host

Echinococcus  
Sparganium  
Coenurus

Both Definitive & intermediate - T. solium.

Paratenic Host - no development of parasite in the host

1) Prawn → Angiostrongylus cantonesis

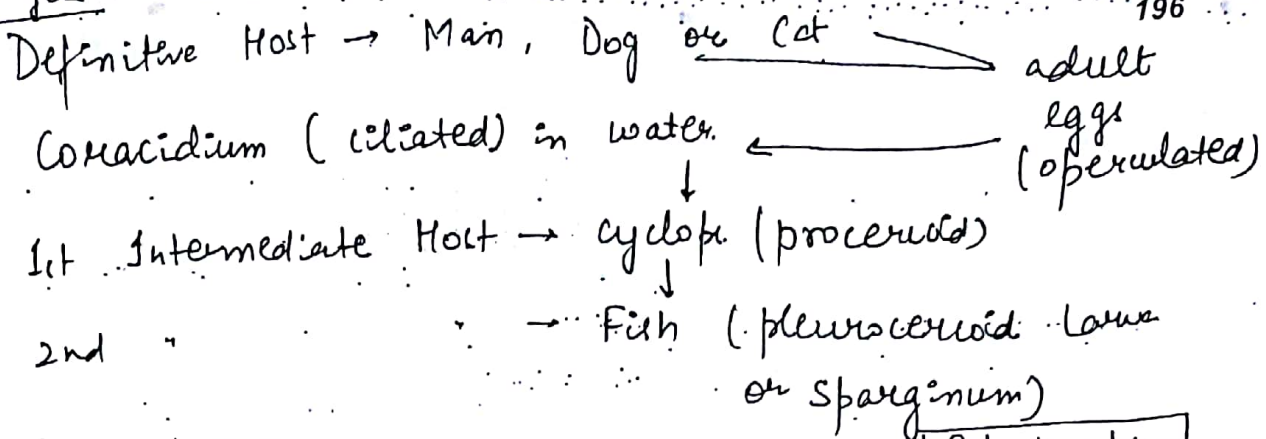
2) Big Fish → pleurocercoid larva of D. latum

3) Fish → Gnathostoma spinigerum

4) Man → pleurocercoid larva of Sparganium Q

## Life cycle DIPHYLLOBOOTHRIUM LATUM.

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infective form

### Pathogenicity:

Asymptomatic Infection - (M/C)

Abdominal Pain

Pernicious Anaemia (absorption of B<sub>12</sub>).

### LARGEST CYSTODE

Δ - Eggs 45-700 (upto 100 μm)

Brown = Operculum at one end.

Knob at another

Eggs of Diphyllobothrium, Fasciola & Fasciolopsis  
>100 μm, operculated, indistinguishable.

### SPARGANUM

Definitive host → Dog, Cat → adult.

↓  
eggs  
↓

Coracidium in water

↓

1st intermediate host - cyclops (procercoid Larva)

→ infective form ← (M/C)

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2nd intermediate host → fish, reptile, amphibians [pleurocercoid  
infective form larva]

Man - pleurocercoid Larva (paratenic form).

## TAENIA

### INTESTINAL TAENIASIS

Infective form - cysticercus (single scolex)

T. Solium

(Pork)

2.5 m

T. Saginata

(Beef)

10 m.

Asymptomatic  
Malabsorption

Δ - scolex → hooks 13 No. in rostellum ⇒ T. Solium

Proglottide

eggs

Bile stained  
straitens



### NEUROCYSTICERCOSIS

Infective form - eggs (contaminated vegetables)  
of T. Solium

↓  
Onchosphere

m/s - (M/C)

↓  
cysticercosis

Brain - epilepsy



Absolute Criteria

↓  
Cysticercus in tissue.

.. by funduscopy

.. by Radiology (Mc)



If cysticercus not seen, then other criteria :-

- a) other radiological signs
- b) Ab detection by ELISA
- c) Clinical evidence
- d) Epidemiological indicators

COENURUS (T. multiceps or T. serialis)

- Multiple scolices
- Coenuri of T. multiceps → found in eye & Brain
- T. serialis → Subcutaneous tissue.

ECHINOCOCCUS (HYDATID CYST)

Ectocyst (Outer cuticular layer)  
acellular

Laminated hyaline membrane  
appears as white of hard boiled egg

Endocyst (Inner germinal layer)  
cellular

vital layer of cyst

~~Gives rise~~ to brood capsules & scolices.

Secretes the specific hydatid fluid - forms outer layer



E. GRANULOSUS → Hydatid Cyst

199

E. MULTILOCULARIS - Alveolar cyst  
(Cyst Metastases)

E. VOGLI - Polycystic Ds

Dog Tapeworm [Definitive Host]

Δ - 1) Casoni Test (anaphylaxis)

2) Ab detection by ELISA

3) CT scan IOC

Screening for Echinococcus  
for E. multilocularis

## DIPYLIDIUM

Infective form = flea harboring cysticercoids [Dog, Cat, Man]  
↓  
Solid cyst = scolex

M/c in children

Asymptomatic M/c

## HYMENOLEPIS

Infective form - egg → cysticercoid → Adults.

One Host (No intermediate Host) Q

Δ - Eggs - 30-40µm

Non-Bile Stained

6 spicules = knobs = HEXACANTH

} similar to  
egg of  
Taenia

# TREMATODES

Q. The infective stage of trematode causing swimmer's itch is  
Cercaria.

Eggs as infective form 200

Hymenolepis

Echinococcus

T. multiceps

serialis

T. solium (NCC)

## SCHISTOSOMA

Separate Sexes

Male - gynaecophoric canal

~~op~~ Non-operculated

No Rediae

Inf. form - Cercaria

↓  
penetrates skin

Life cycle

## OTHERS

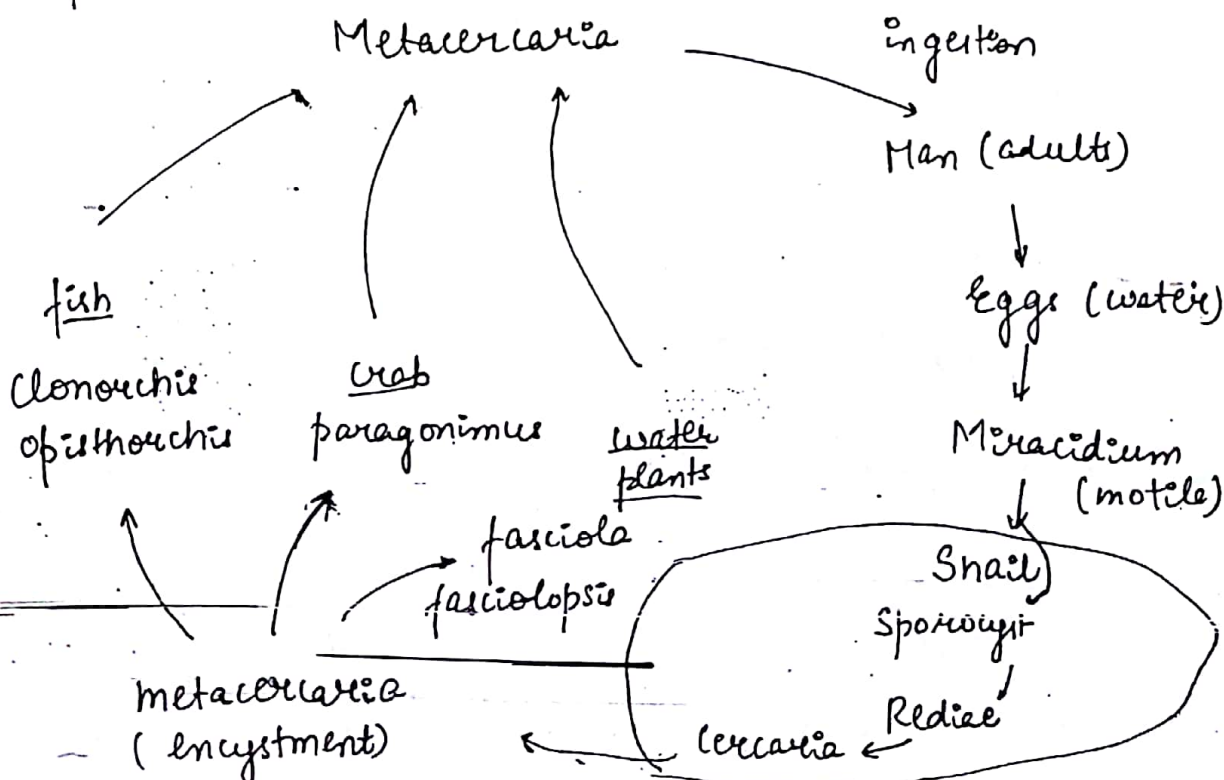
Hermaphrodite

Operculated

Rediae -

Metacercaria

↓  
Ingestion



All Trematodes are oviparous

Q 201

Hepatomegaly  $\rightarrow$  egg granuloma

Katayama Syndrome  $\rightarrow$  oviposition, a serum sickness like illness.

Fibrosis (Symmer's)  
due to Th1 response

Swimmer's itch [cercarial dermatitis]  
in *S. mansoni*, *S. japonicum*.

*S. Haematobium*

Egg - 100-150  $\mu$ m, non-operculated

*S. Haematobium* - terminal spine.

Ca Bladder.

*S. Mansoni* - Lateral spine

*S. Japonicum* - spine inconspicuous

CLONORCHIS OPISTHORCHIS

Only egg of Trematode  $< 100 \mu$ m = 15-30  $\mu$ m  
operculated  $\bar{c}$  shoulder.

Cholangiocarcinoma

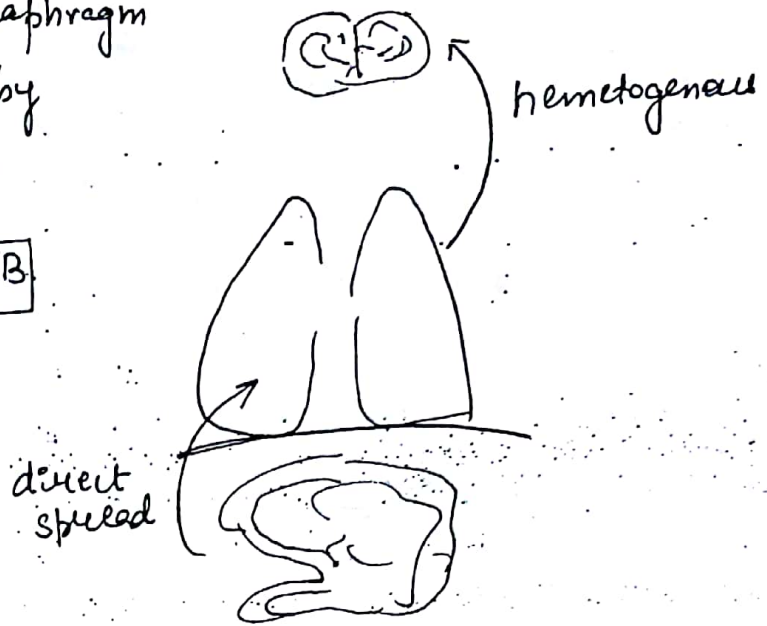
PARAGONIMIASIS

## PARAGONIMUS

202

It directly pierces Diaphragm  
But spread to brain by  
haematogenous spread

Paragonimus Mimic P.TB



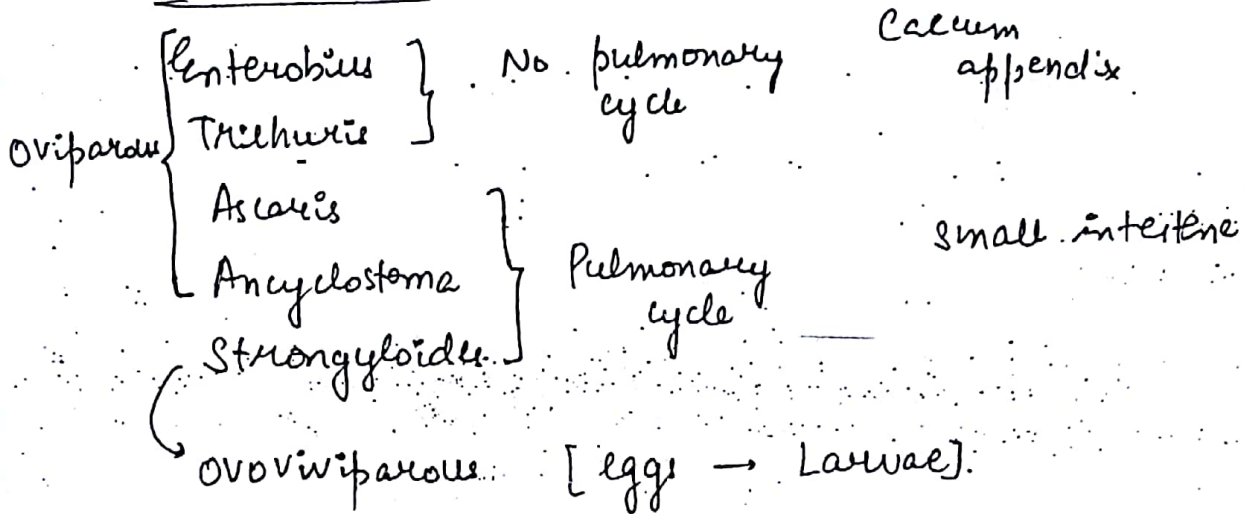
Δ - Golden Brown eggs in the sputum.



# NEMATODES

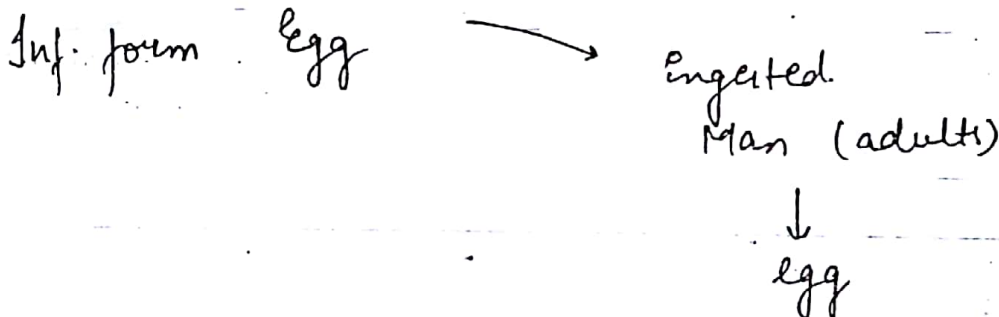
203

## INTESTINALS



Larvae In Stool → Strongyloides

## ENTEROBIUS — (Pin worm) —



Adults — Pointed / sharp ends → Pruritus M/c symptom

Δ Scotch tape (NIH swab)  
↓  
Perianal Region.

Egg :- 70 μm  
Non Bile Stained  
Plano-convex Δ

## TRICHURIIS (Whip worm)

204

Asymptomatic Infection

Anaemia

Rectal Prolapse

Δ - eggs in stool

70-80 μm

Bile stained

Barrel shaped

Bipolar plugs

## ASCARIS

Infective Form - egg & rhabditiform larvae



Larvae

(penetrates into circulation)



Heart

↓  
Lungs

small Intestine  
(adults)

Epiglottis  
(swallowed)



Adults - Fecundity ↑ (No. of eggs laid/worm/day)  
(2.4 lakhs)

Cause intestinal obstruction.

Larvae → Loeffler's syndrome

Δ - adults (male are shorter & have curved ends)

egg

50-60 μm

Bile stained

Rugosity



Non-Human Ascaris worms

Toxocara canis M/c

205

" cati



Visceral Larva migrans

## ANKYLOSTOMA

Infective form - Filariiform Larvae

↓  
penetration of skin

Adults - 0.2 mL of blood/worm/day

Anaemia

Larva - Loeffler's Syndrome

Δ eggs - 50-60 μm  
Non bile stained  
& blastomeres



## Non-Human Ankylostoma

A. Braziliensis → Cut. Larva Migrants  
M/c "creeping eruptions"

## STRONGYLOIDES

Inf. form - filariiform Larva  
↓  
penetration of skin

Parthenogenic female - lay eggs w/out males  
fertilised

Indirect development in soil → ♂ = ♀

Dermatitis LARVA CURRENTS - larvae migrate @ 1 cm/hour.

Δ ① Bermann Funnel technique

206

② culture by Harede - Moore Filter paper technique / agar plates.

Filariform Larvae → sharp @ side



Rhabditiform Larvae - Blunt 1 end

### TISSUE NEMATODES

Filarial worms

Trichinella spiralis

### FILARIAL WORMS

Inf. form → 3<sup>rd</sup> stage Larva  
↓ mosquito

Tissue

Signs + symptoms → due to adult  
except ONCHOCERCA.

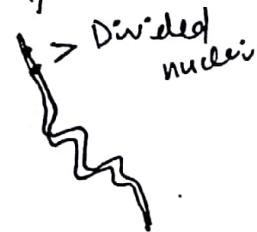
Δ - Microfilariae in blood.  
except onchocerca

### BRUUIA MALAYI

Fragmented Nucleus in tail end of microfilariae

Nucleus - Blue

Cytoplasm - Pink



### W. BANCROFTI

Nuclear material do not extend to the tip

### LOA - LOA

Nuclear material extends upto loa loa



## MANZONELLA

Unsheathed.

Nuclear material upto tip

207

## ONCHOCERCA

Simulium (deer fly)

↓  
Inf. form Larva

↓  
adults in tissue nodules  
over bony prominences.

also causes RIVER BLINDNESS

Δ. adults in tissue

culture of larvae from skin snips

ANGIOSTRONGYLUS CANTONENSIS (rat lungworm)

eosinophilic meningitis

Infective form - Ingestion of 3<sup>rd</sup> stage in mollusks.

visceral larva migrans in Brain - M/c cause is this.

Angiostrongylus costaricensis

Abdominal angiostrongyliasis M/c.

Symptoms mimic appendicitis.

## ANISAKIASIS

Anisaki Simplex + ~~Pseudoterranova~~ decipiens

3<sup>rd</sup> stage Larva in fish.

Surgical Resection.

## Gnathostoma spinigerum

208

3<sup>rd</sup> stage Larva in fish or poultry

Eosinophilic meningoencephalitis

Migratory cutaneous swellings of the eye & visceral organs.

Surgical Resection.

## TRICHOSTRONGYLUS

Infection - Ingestion of Larva (vegetables)

Ingest far less blood than hookworms

Asymptomatic (M/C)

Heavy infections - anaemia + eosinophilia

Stool exam

Eggs resemble hookworm eggs but are larger.  
(85 by 115  $\mu$ m)

## TRICHINELLA SPIRALIS

Infected form - Encysted Larvae in pork or polar bear

↓  
adults in intestinal mucosa

↓  
migration + encysted larvae  
cause signs + symptoms

Δ - Eosinophilia

↑ CPK

Ab detection

M/s Biopsy (at the tendon insertion)

Lemon Sign (nurse cells)

Bachman Intradermal Test

209

M/s involved → EOM, Biceps Jaw, Diaphragm.

Larval Load -

< 10 larvae/gm of tissue

↓  
asymptomatic

> 50

→ fatal.

Egg Load → Chandler's Index

7300 → Major Public Health problem  
seen in Hookworm

### AUTOINFECTION

C - *Cryptosporidium*, *Capillaria philippinensis*

H - *H. nana*

E - *Enterobius*

S - *Strongyloides*

T - *Tenias solium*

# MYCOLOGY

210

Cell Wall - Chitin  
Mannan  
Glucans

Cell Membrane - Ergosterol  
 $\beta$ -glucan assay - all fungi except *Cryptococcus*.

SDA

Isolates  $\rightarrow$  Lactophenol  
cotton Blue.

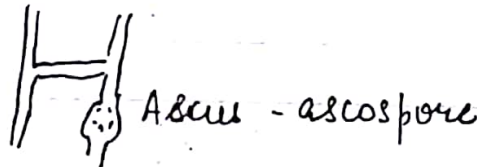
Calcofluor white  $\rightarrow$  fluorescent

Classification. (Sexual reproduction)

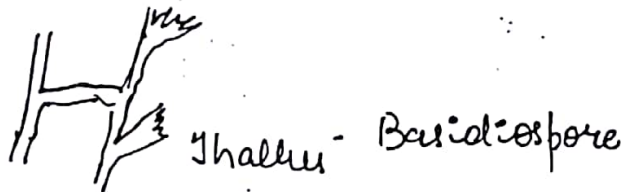
zygomycete



Ascomycete



Basidiomycete



Deuteromycete No sexual spore

'Fungi Imperfecti'

Morphology

Yeast - *Cryptococcus*

Yeast like - *Candida*

Dimorphism -  $\begin{cases} 37^\circ\text{C} & \text{Yeast} \\ 25^\circ\text{C} & \text{Mould} \end{cases}$  Endemic



Ph I Sporothrix schenckii - Himalachal. [Rose Gardener's D]  
Penicillium marneffii - Manipur. 211

Histoplasma → Eastern } North America  
Blastomycosis }  
Coccidiomycosis → western }  
Paracoccidiomycosis → South American

Moulds. Reif

## OPPORTUNISTIC FUNGAL INFECTIONS

M/c - Candida

### CANDIDA

Endogenous —

↓  
CMI ↓ → mucocutaneous

Neutrophil ↓ → invasion


↓  
True & Pseudohyphae

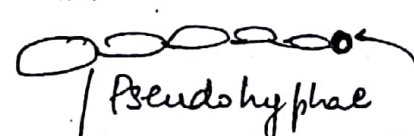
True Hyphae → apical elongation when candida is  
[Raynaud Braun phenomenon] grown in serum for 2 hours

Pseudohyphae → failure of daughter buds to separate

↓  
Seen in Corn meal Agar

[Nutritionally Deficient media]

Germ Tube →  HYPHAE

 Pseudohyphae

constrictions

Chlamydo  
conidia.

Candida  
Albicans

Non albicans

Germ Tube +

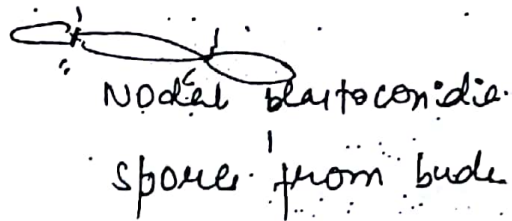
~~Chlamydoconidia~~ +  
Chlamydoconidia

8-7

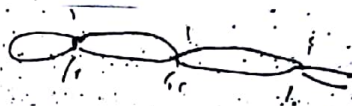
C. Dubliniensis - non-albicans  $\Rightarrow$  can produce  
germ tube, chlamydoconidia

### NON-ALBICANS

C. Tropicalis



C. parapsilosis

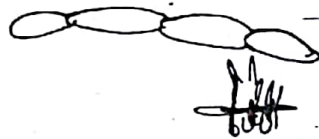


(B) Node + internode

C. Glabrata  
(mucoid)

No pseudohyphae

C. Krusei



Cross matchstick

### CRYPTOCOCCUS

It causes infect<sup>n</sup> in HIV pt.

Virulence factors:

- (1) Sialic acid
- (2) melanin
- (3) Urease
- (4) Superoxide dismutase
- (5) Mannitol fermentation
- (6) Capsule

(7) Mating Types

1<sup>o</sup> Infection  $\Rightarrow$  LUNGS  $\longrightarrow$  then Brain.

Δ - culture in Niger Seed Agar → Brown mould colonies  
India Ink.

213

Aq Detection by Latex Agglutination.

IgA + IgG<sub>2</sub> ⇒ protective against capsule

~~Obtuse~~ ZYGOMYCELES

obtuse ∠



Nodal - Rhizoids



Absidia  
Internodal rhizoids

Mucor - No rhizoids

Angio invasive → Rhinocerebral mucormycosis  
↑ mortality

R/F - Diabetes ketoacidosis

Desferrioxamine R

Δ - Profuse Growth → Lid thrower.

ASEPTATE IRREGULAR BROAD, ribbon like hyphae

Branching at OBTUSE ANGLE.

ASPERGILLUS

Fumigatus → Invasive (↓ neutrophils)

Flavus → Keratitis

Niger → Otorrhoea

ACUTE ANGLE DICHOTOMOUS BRANCHING  
'V' forms



## PENICILLIUM MARNEFFI

Umbellated lesion. → like molluscum contagiosum  
seen in HIV pt

Δ - Septate yeast → Binary Fission

Red Pigments



Broom stick

## PNEUMOCYSTIS JIROVECHII

cause Interstitial Pneumonia (Non productive cough)

AIDS - induced sputum

HIV - BAL

Δ - can't be cultured

Gomori methamine silver → cyst wall

Toluidine Blue →

Giemsa → sporozoites (8 in NO)

Best IFA → Best Technique

## Fungal Infection In Immunocompetent

Dermatophytes (MIC)

↓  
Keratinophilic



	Macroconidia	Microconidia
Trichophyton (skin, hair, nail)	pencil shaped few	plenty 215
Microporum (skin, hair)	spindle (boat) plenty	few
Epidermophyton (skin, nail)	clavate (club)	No
Mentagrophytes (Trichophyton)	→ Hair Perforation (+) urease (-)	—

### T. VERSICOLOR

Malassezia globosa (M/C) , furfur  
↓  
lipophilic

Δ culture — SDA ± olive oil  
Spaghetti x Meat ball

TPN Rx → Invasive  
(± lipids)

### SPOROTHRIX SCHENCKII

Thorn prick 'Rose Gardener's Ds'  
Lymphatic spread → series of ulcers

Δ - Cigar shaped yeast  
Rosette like conidia in SDA  
'Splendore Hoeppli phenomenon'  
(asteroid)

→ Cutaneous zygomycete

→ Blastomycetes

## CHROMOBLASTOMYCOSIS

Coloured jungle (Pigmented)

216

Δ - Sclerotic Body → Brown septate Yeast appearing like "copper coin".

## 'BOOMERANG CONIDIA' CURVULARIA

Dermatiticous

1) Curvularia

2) Alternaria

3) Bipolaris

4) Cladophialophora

5) Exophiala

6) Fonsecaea

7) Madurella

8) Scedosporium

9) Scytalidium

10) Wangiella etc.

C/F - Verrucose cauliflower like lesion.

## MYCETOMA

C/F

Swelling

Sinus

Granuloma

## RHINOSPORIDIUM SEEBERTI

- PROTOZOA

- Polyps R/F → Pond Bathing

- Δ - 10% KOH → spherules c Endoscope


# SYSTEMIC FUNGI

Infective form  $\Rightarrow$  Arthroconidia  $\rightarrow$  spores in Hyphae 217

$\Downarrow$   
Pulmonary Lesions  
TB like

$\Delta$  - Blastomycosis  $\rightarrow$  Broad Based budding

Paracoccidioidomycosis  $\rightarrow$  multiple budding yeast

Mariener/Pilot wheel 

Coccidioidomycosis - Barrel shaped arthroconidia  
Valley Fever

Desert Rheumatism

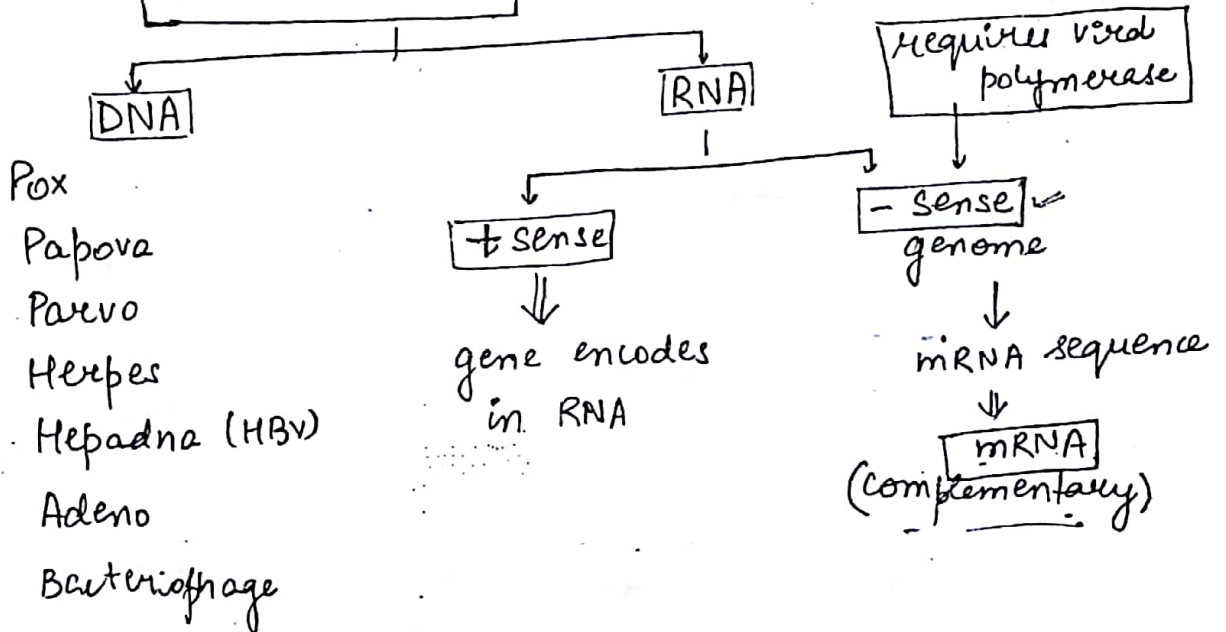
Spherules (tissue) 

Histoplasma Capsulatum  
found in Bats.

$\rightarrow$  Tubercular ~~like~~ projections  
on 'spores'.



## VIROLOGY



## Non-Enveloped Virus

⇒ Released by Lysis

218

### DNA

Parvo

Adeno

Papova

### RNA

Astro

Picornia

Reo

Calci

HAV

HEV

SP Cancer. Ho. RAE

enveloped virus released by budding.

## Segmented RNA viruses

Genetic re-assortment occurs.

They can show genetic shift

B - Bunya virus (3 segment)

I - Influenza virus (8 segments)

R - Rota virus (11 segment)

A - Arena virus (2 segment)

## DNA VIRUSES

### PARVO B<sub>19</sub>

Smallest 20nm

affinity to immature RBC

Immuno competent child.

(5<sup>th</sup> DS)

Erythema  
Infectiosum



Ab excess

↓  
Immune complex

↓  
Vascular  
Damage

← (slapped cheek)

Immuno competent Adult - Ab excess → polyarthropathy

Sickle cell

→ Ab  
response

→ Aplastic (Transient)  
crisis



Immunocompromised - No Ab response → Pure Red Cell Aplasia (PRCA) 219

⊕ → fetal liver, spleen, kidney ⇒ Hydrops Fetalis.

Δ - Ab detection

qPCR

Quantitative PCR (Real Time PCR) (Best)

↓  
Taq man assay

**PAPOMA**

POLIOMA = JC virus → Progressive Multifocal leucoencephalopathy (PML)

BK virus → Kidney Infection

PAPILLOMA

↓ warts

Ca Cx → **E protein** → virulence

↓  
Suppress the tumour suppressor genes

**L protein** → serotyping

6, 11, 16, 18, 31, 45, 47, 52, 58  
Low Risk      50%      80% infection      High Risk

VACCINE → Cervarix (16, 18)

Gardasil (6, 11, 16, 18)

Priz Gardasil 9

Δ of Ca Cx = Koilocyte → cells = pyknotic nucleus + perinuclear halo

## Pox

220

Molluscum Contagiosum

↓  
Pearly white umbilicated nodular lesions  
self limiting  
direct contact

Δ - Molluscum Body (IB).

Bollinger Body → fowl pox } Inclusion Body  
Guarnieri Body → vaccinia }

DNA virus → Intracellular except Pox  
RNA " → Intracytoplasmic except influenza, HIV

## HERPES

HHV 1 } α virus.  
HHV 2 } ↓  
HHV 3 } epithelial cells

HHV 5 } β virus  
HHV 6 } ↓  
HHV 7 } Glands

HHV 4 } γ virus (oncogenic)  
HHV 8 } ↓  
Lymphocytes

HHV 1  
Stomatitis  
Keratitis  
Encephalitis  
[Temporal → frontal]  
less

HHV 2  
Genital infection  
Aseptic meningitis.  
More virulent

Anti HSV → non protective

due to  
trivial  
exposure

1° Infection → Latency → Reactivation.  
↓  
EPISOME (integration of viral DNA to host chromosome)

A - ~~clausson~~ Tzanck Smear → Giemsa

221



↓  
Multinucleate Giant cell

Eosinophilic IB (~~inter~~ intranuclear)



Cowdry A

② Ab detection

③ PCR → IOC

HHV 3 - V-Z virus

Chickenpox → Lesions in crops

Lifelong Immunity in case of Chickenpox

Shingles → Reactivation from dorsal n/v root ganglion.  
of Trigeminal & Sacral n/v.



affect T<sub>3</sub> to L<sub>2</sub> u/l

Trigeminal, Facial  
(Ophthalmic Div.)

Ramsay Hunt Syndrome.

Ant  $\frac{2}{3}$ <sup>rd</sup> of Tongue

middle ear lesions

Bell's Palsy.

Congenital V-Z Syndrome

Scarring of Lesion (skin)

Hypoplasia of limbs

Chorioretinitis.

A - Tzanck

Ab detection

PCR



Vaccine - 'oka strain'

222

Passive - Vz Ig.

HHV-4 → EBV

Reservoir → memory B cells

① Infectious Mononucleosis → False ⊕ serological Test  
(Kissing Disease)

↓  
Paul Bunnell Test (Heterophile agglutination test)

↓  
Sheep RBC

Differential Ab adsorption Test

	Guinea Pig Kidney	OX RBC
Normal Serum Ab	adsorbed	⊖
Foxsman Ab (after serum Rx)	adsorbed	adsorbed
IM	⊖	adsorbed

② Oral Hairy Leukoplakia

white plaque under Tongue. (self limiting)

children, HIV pt

↓  
bleeds

③ Malignant - Nasopharyngeal Ca  
Burkitt's Lymphoma  
Hodgkin's  
NK/T cell  
B cell Lymphoma

Lymphoproliferative Ds  
Adenoma



## HHV-5 CMV

223

enlargement of cells

ubiquitous

Secreted in all body secretions



Retinitis in HIV ⊕ < 50 CD4

Inclusion Body Ds - children.

Transplantation

Δ ① Owl Eye I.B.

② Ag Detec<sup>n</sup>

③ PCR

HHV-6 - 6<sup>th</sup> Ds - ROSEOLA INFANTUM  
or  
Erythema Subitum

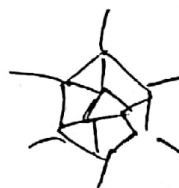
HH7 → ↓ CD4 in HIV

HH8 → Kaposi's Sarcoma

## ADENO VIRUS

Apical Fibrils

"space objects"



Serotype

8, 19, 37

→ Epidemic Keratoconjunctivitis

11, 21

→ Acute haemorrhagic  
cystitis

40, 41

→ Infantile Gastroenteritis

1, 2, 3, 5

→ Resp. Infections

Vaccine :

Live Non-attenuated Vaccine

→ Administered through oral route

Δ - ① Ab detection

② Culture in cell lines (human origin)

224

②

MELA/ HEP

Calx

Ca Larynx

Barophilic I.B. [Cowdry B]  
(intracellular)

## RNA VIRUSES

### Infantile Gastroenteritis.

Rota Virus M/c → ds RNA

vomiting followed by diarrhoea

family - ~~Reo~~ Reo

11 segments.

Vaccine - VPG + VP7.

↓  
Intussusception.

Δ - VPG Ag detection by ELISA in stool

CALCI

Noro (Norwalk) → M/c in adults + children

Sapo

ASTRO

Toga

Adeno Type 40, 41 - DNA Virus. (only)

Δ - can't be cultured in  
EM.

# ORTHOMYXO

## Influenza

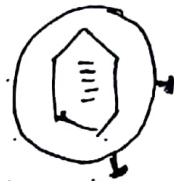
Type A

8 segments HUMAN

B

C → 7 segments

Based on nucleocapsid protein.



H haemagglutinin 18  
N neuraminidase 9

Envelope Glycoprotein.

Δ - RT PCR.

Sample - nasopharyngeal swab.

## AVIAN INFLUENZA

Reservoir.  $H_5N_1$

↑ virulent

NO person to person Transmission.



Migratory Birds (Reservoir)

$H_5N_1$

water

poultry

death

[culling]



PIG (swine)

Receptor for all influenza virus

amplifier

Genetic Reassortment

New virus

→ Pandemics

Antigenic shift (change in H.)

Mutation → epidemics → antigenic drift.

$H_1N_1$  → 1918 Spanish flu

$H_2N_2$  → 1957 Asian

$H_3N_2$  → ~~1968~~ 1968 Hong Kong

Pig → Reservoir.

Von Magnus phenomenon

↑ titre (haemagglutinin)

↓ infectivity

Δ - RT PCR.

### PARAMYXO

RSV → Bronchiolitis

Mumps → Parotitis - aseptic meningitis M/C  
orchitis

Vaccine - Jeryl Lynn Strain.

Measles → Fever & Rash (IP - 14 days)

↓  
Behind ears (1st)

KOPLIK'S SPOT → Opp. 2<sup>nd</sup> Molar (lower)

M/C complication - otitis media

M/C CNS " - Post measles encephalitis  
(autoimmune)  
in 1 year.

SSPE - Rare complication

↓ 5-25 years.

due to defective virus. (spongiform encephalitis)  
Prion proteins

Δ - Ab detection (after 7 days of onset of Rash)

PCR - (nasopharyngeal swab)  
in 5 days.



# Vaccine - Edmonstan Zagreb

227

## RUBELLA

German Measle

Vaccine → RA 27/3

Adults → Exanthematous Rash / Keratids.

### Congenital Rubella Syndrome

Cataract

Deafness

Heart Defects

Δ - Ab detect<sup>n</sup>

⊙ 1st Trimester - is exposed to her friend suspected of Rubella - What next?

susceptibility

⊙  
♀

♀ → diagnose

If friend have IgM +ve,  
then check for IgG in ♀

IgG +ve → no worry

IgG -ve → come for next  
month

IgM +ve → abortion.

a) IgG

b) IgG, IgM

c) IgG

d) -

IgM

IgM.

## ARBOVIRUS

arthropod virus

Flavi Virus

Bunya "

Toga "

→ mosquito + Ticks.

✓ Powassan

✓ Russian spring summer

✓ Kyasanur forest ds

✓ CCHF (Crimean Congo HF) haemorrhagic fever

DENGUE

5 serotypes

DF

→ DHF/DSS

Ab enhancement

Immune complex

\* Factors affecting Haemorrhage :-

1) Repeat infection w/ different serotype

2) Sequence of infection. Type 1 followed by Type 2

3) Serotype 2

4) Whites

5) &lt; 12 yrs.

6) Female

→ Malnutrition → protective

Δ - IgM Capture ELISA

NS1 Ag (in 5 days)

ZIKA

\* Microcephaly in newborn.

JE → pig → amplifying host

Δ - Ab detection

Vaccine - SA-14-14-2 (Live) - 1 dose

229

↓  
tissue culture

CCHF (Crimean Congo Haemorrhagic Fever)

- Case reported in Gujarat, Rajasthan.

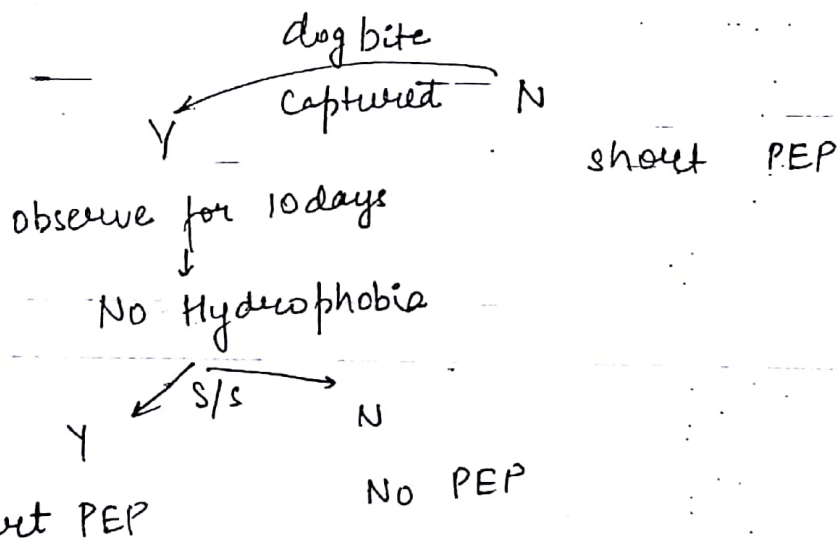
Δ - Ab detection

✓ PCR

Rhabdovirus

Bullet shaped

3 mm/hr or 230 mm/day [Rate of spread].



↓  
antemortem (pt) → skin from  
nape of neck.

↓  
Immunofluorescence

Postmortem (behead dog) → Brain.

↓  
Sellar stain

↓  
Negri Body → + Hippocampus  
cerebellum

AFP

Δ - stool culture (2 samples)

↓  
sequencing → Type 1 or 3 or VDPP

HEPATITIS

HAV

(Picornavirus)

feco-oral route → acute infect

↓  
fulminant in adults  
outbreak

Δ - only hepatitis virus cultured in cell line

IgM Anti-HAV

↓  
one serotype & 4 genotype

HEV

(Hepe virus)

feco-oral route → acute infect

↓  
fulminant in pregnancy  
outbreak

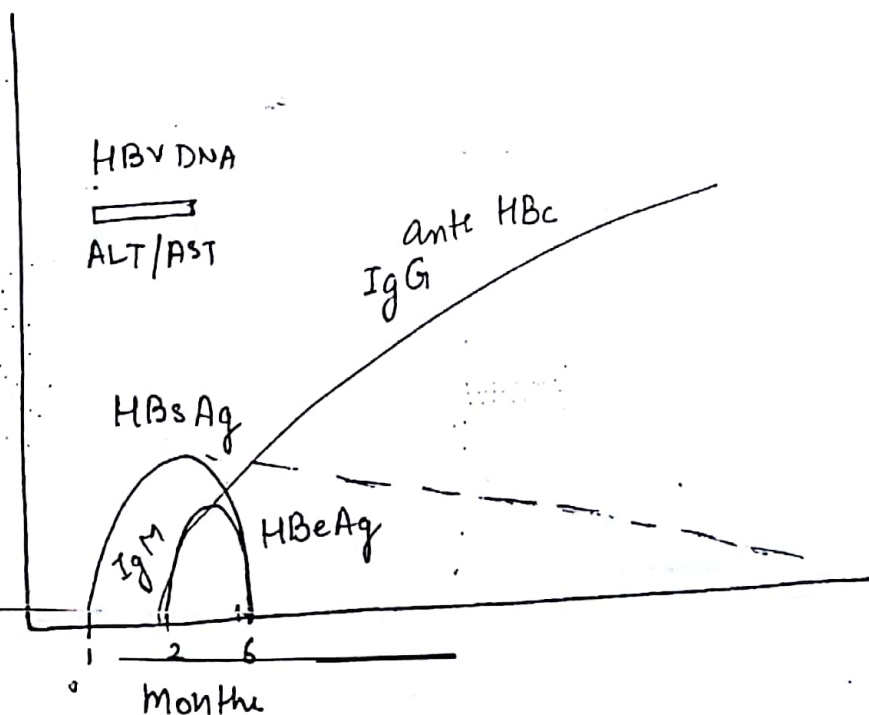
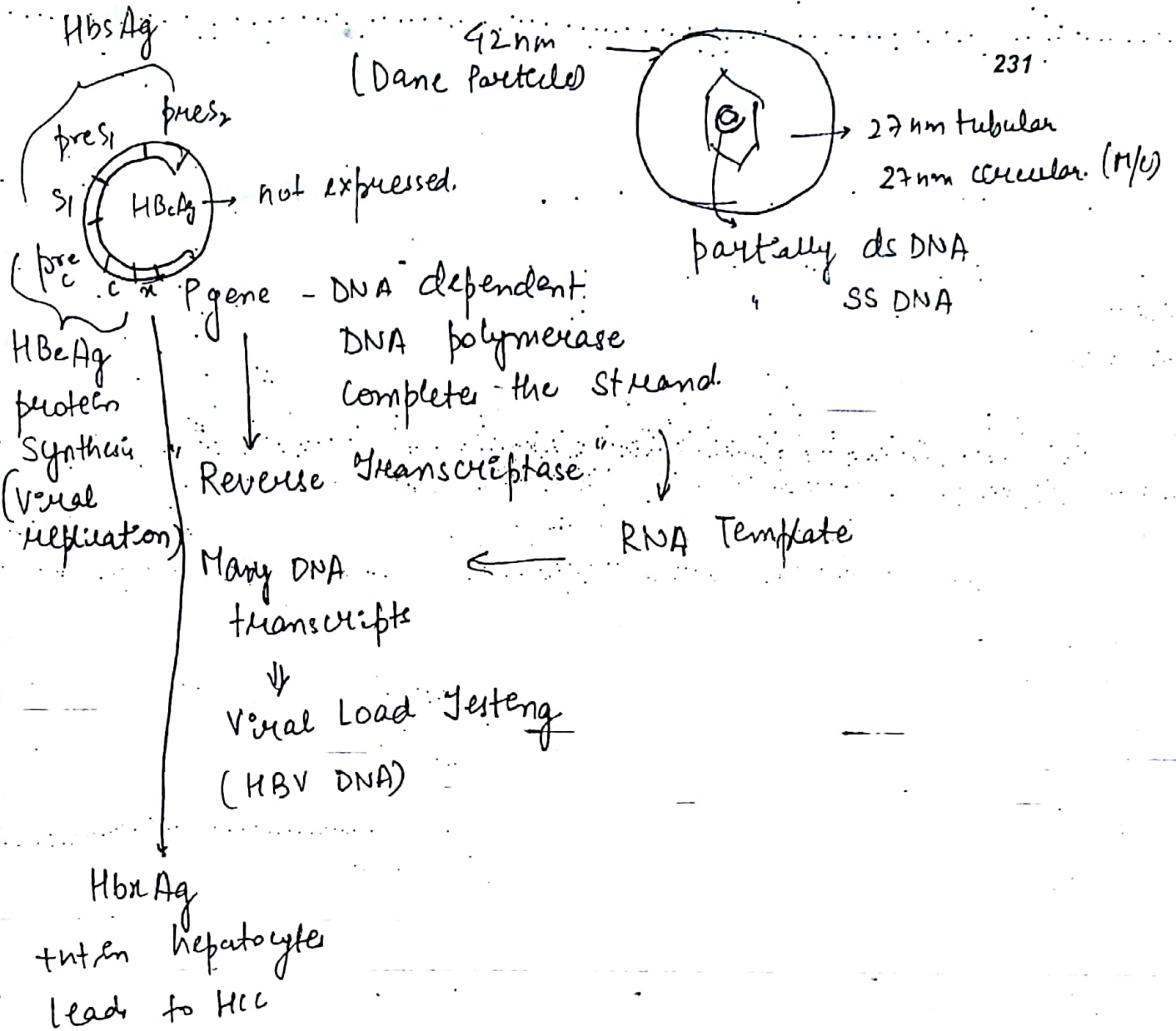
Blood Transmission (rarely documented)

Δ - IgM anti HEV

HBV

(Hepadnavirus)





Acute Hep B infect in adult  $\Rightarrow$  usually resolve <sup>232</sup>

Vertical Transmission  $\rightarrow$  chronicity  $\uparrow$   
(M/c)

Best Marker of Acute Hep B  $\Rightarrow$  IgM Ante HBc

Chronic active supercarrier  $\rightarrow$  Infections . Require Rx

$\Downarrow$   
IgG ante HBc , HBV DNA

Chronic persistent  $\rightarrow$  IgG ante HBc <sup>(+)</sup> HBV DNA <sup>(-)</sup>

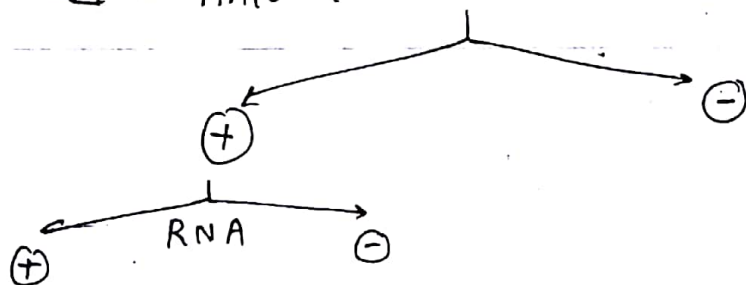
HCV flavivirus.

Chronic infect  $\uparrow\uparrow$

acute  $\rightarrow$  No signs/symptoms.

unsafe inject  $\rightarrow$  (M/c)

$\Delta$  - Ante HCV



Rx

Sofosbuvir +  
daclatasvir

3 months

genotyping not Req

Rebavirin + IFN  $\rightarrow$  genotyping

Index Type 3  $> 1$

HIV

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Co-infect<sup>n</sup> → IgM Anti HBe

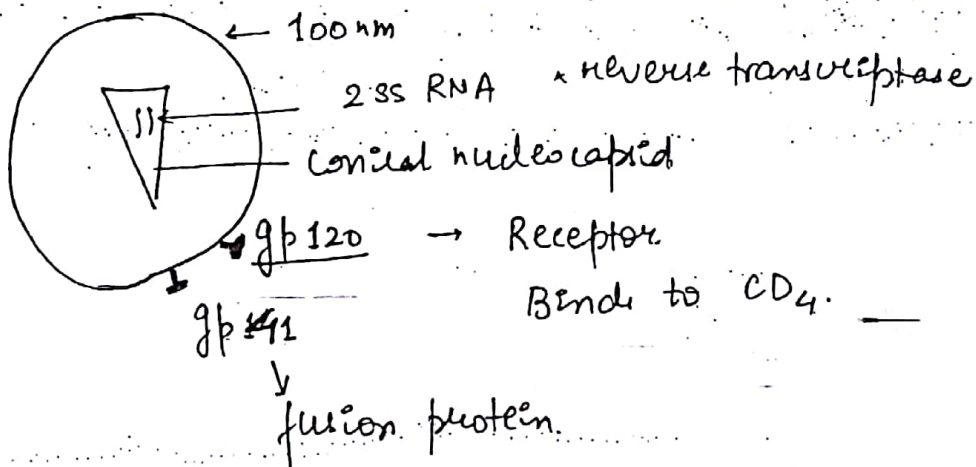
Super infect<sup>n</sup> → IgG anti HBe

fulminant

↑ incidence of fulminancy - 20%

H/c fulminancy → HBV

HIV



Co-receptor on the host cell

(CCR5)

CXCR4

(R5 virus)

(X4 virus)

M tropic

T Tropic

Monocytes/  
macrophages

Lymphocyte

↓  
Resistance

HIV 1

HIV 2

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M N O  
Major  
Human

West Africa  
Intense  $\oplus$  to NNRTI

Subtypes - C - India

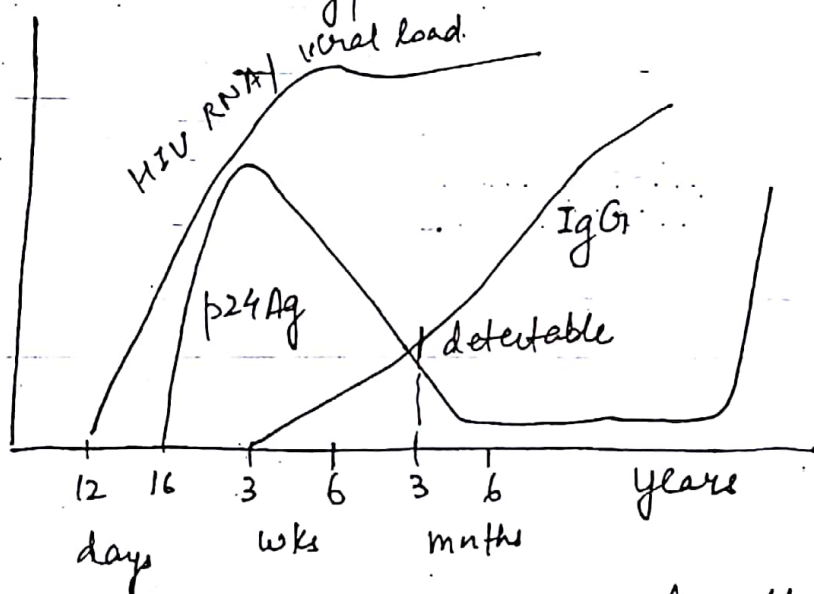
Env gene is conserved between HIV 1 & 2

gag & pol share 90% homology

env Ag. used for diagnosis

gp 120 : gp 41 - HIV 1

gp 36 - HIV 2



Recent H/o exposure p24 Ag  $\rightarrow$  day 16 to 3 months

$\Delta$  of HIV  $\rightarrow$  Ab detection

Most sensitive  $\rightarrow$  4th generation ELISA

detects both p24 Ag, HIV Ab.

$\downarrow$  3 tests

Confirmation  $\rightarrow$  Western Blot



Δ in children - DNA PCR Q.

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"dried blood spot"

### Disease Monitoring

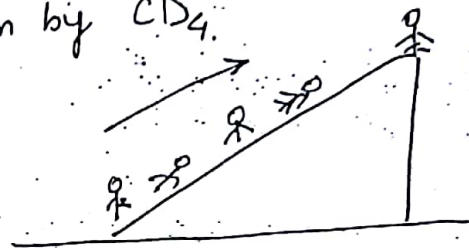
CD<sub>4</sub> → opportunistic Inf<sup>n</sup>, treatment

Viral Load → Prognosis

Present Immune status given by CD<sub>4</sub>.

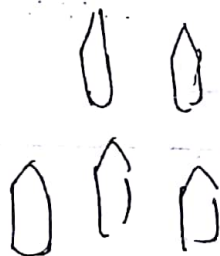
Rapid progressor (synergic)

Slow "



### SEROLOGY

Ag - Ab Rxn.



If Ab excess → PROZONE

False -ve Rx

If Ag excess.  
POST ZONE



zone of equivalence

Serological Test → dilution (2 fold serial)  
of serum

Titre → Highest Dilution at E Rxn is seen.

## PPT<sup>n</sup>

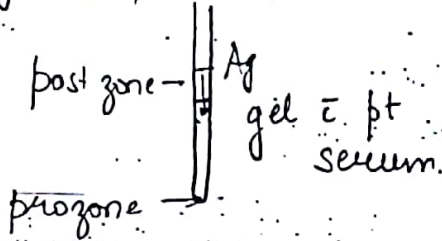
## Agglutination

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Soluble Ag binds to  $\bar{c}$

Ab to form soluble ppt

Single diffusion in one dimension Oudin procedure



Double diffusion in one dimension Oukley Fulthrop procedure

Single diffusion in two dimensions Radial immunodiffusion

Double-diffusion in two dimensions Ouchterlony procedure  
eg. Elek gel's electrophoresis

Quantitative  $\rightarrow$  Rocket Electrophoresis

## Agglutination

Particulate Ag binds to Ab to form visible clumps



Passive Agglutination  $\rightarrow$  Particles to detect Ab

Reverse

$\rightarrow$  particles to detect Ag

## Complement Fixation

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Detects Ab.

ELISA is used nowadays → as it detects Ag + Ab

ELISA large no. of samples ~~test~~ tested at 1 time

Direct ELISA

detects Ag  
specific

Indirect ELISA

detects Ab  
sensitive

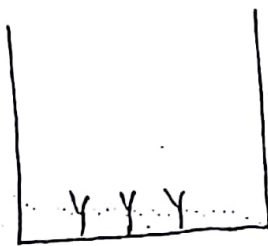
Competitive ELISA

— detects Ab → highly specific

Capture ELISA

detects isotype of Ab

using monoclonal Ab → isotypes are captured i.e.  
eg. Dengue IgM.



mAb for IgM.

add Dengue Ag + Ab + antigen

↓ now add substrate  
+ enzyme

colour change

# Immunofluorescence

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FITC dye

fluorescein isothiocyanate.

Chemiluminescence (CLIA)

↳ light emitting particles.

10-100 times more sensitive ELISA.

## Obligate Intracellular Parasite

CRV CM PTL

Chlamydia

Rickettsia (Ehrlichia, anaplasma)

viruses

Coxiella Burnetii

Cryptosporidium parvum

Mycobacterium leprae

Plasmodium sp.

Pneumocystis jirovecii

Toxoplasma gondii

Trypanosoma cruzi

Leishmania Donovan

## Facultative Intracellular Parasite

MBBS CRV for NHL

Mycobacterium

Bartonella henselae

Brucella

Salmonella Typhi

Cryptococcus neoformans

Rhodococcus equi

Yersinia

Francisella tularensis

Nocardia

N. meningitidis

Histoplasma capsulatum

Coccidioides immitis

Legionella